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United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Plant Protection
and Quarantine

Biological Control
Operations

BIOLOGICAL CONTROL OF CEREAL LEAF BEETLE PROJECT MANUAL

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INTRODUCTION

Orientation to the Cereal Leaf Beetle (CLB) Biological Control Project

History

Like many of our agricultural insect pests, the CLB was accidentally introduced into the United States. This pest of small grains was first found in Michigan in 1962. From there it rapidly spread throughout most of the Midwest and into the northeastern part of the country. In response to the damage caused by this pest (see Appendix 1), the United States Department of Agriculture (USDA) began efforts in 1963 to locate suitable natural enemies in the pest's native range in Europe. Several species of natural enemies were imported into the United States where they eventually became established and successfully controlled the pest. After establishing and distributing the natural enemies throughout the range of CLB, APHIS ended the project in 1978. At that time the pest was being controlled biologically by the complex of parasites.

Since 1978 CLB has continued to expand its range (see *Figure 1* for current distribution). For many years the parasite complex also moved with the pest. In recent years though, this pest moved into several southeastern States and caused economic damage. In the mid-1980's, CLB was also found in Utah, then in Montana. The western populations apparently moved without a simultaneous movement of the natural enemies. As a result of these recent developments, APHIS reentered the CLB Project in 1993.

Goal and Objectives

The project goal is to implement and provide coordination in a cooperative reintroduction and colonization project against CLB, using exotic natural enemies in select States. Objectives of the project include the following:

- Importing and releasing new strains of natural enemies from Europe that are better suited to the expanded range of the pest
- Establishing field insectaries which will serve as sites for rearing the new strains in the United States, and later as source locations for natural enemies for distribution to problem areas
- Collecting the now indigenous strains of natural enemies from sites in the Eastern United States
- Releasing natural enemies in the Western United States and along the edge of the expanded range in the eastern part of the country
- Evaluating the establishment of existing natural enemies in select States
- Rearing natural enemies in the laboratory to support field releases

Sections

The following instructions serve as guidelines for field activities. These guidelines include the following sections:

- Introduction
- Fall Collection of Cereal Leaf Beetle Adults
- Spring Collection of Cereal Leaf Beetle Adults
- Collection of Parasitized Cereal Leaf Beetle Eggs
- Collection of Parasitized Cereal Leaf Beetle Larvae
- Establishment Evaluation
- Field Insectary Establishment and Maintenance
- Insectary Monitoring
- Releases of Natural Enemies

Who's Involved The Project Leader is Michael Bryan, who is located at the National Biological Control Laboratory, PPQ, APHIS, in Niles, Michigan.

Cooperators include PPQ line personnel, State departments of agriculture personnel, university researchers, and Extension Service personnel. State project coordinators are generally State plant health directors (SPHD's) or officers-in-charge (OIC's) at designated PPQ locations.

Cereal Leaf Beetle

Prepared by Niles Biocontrol lab

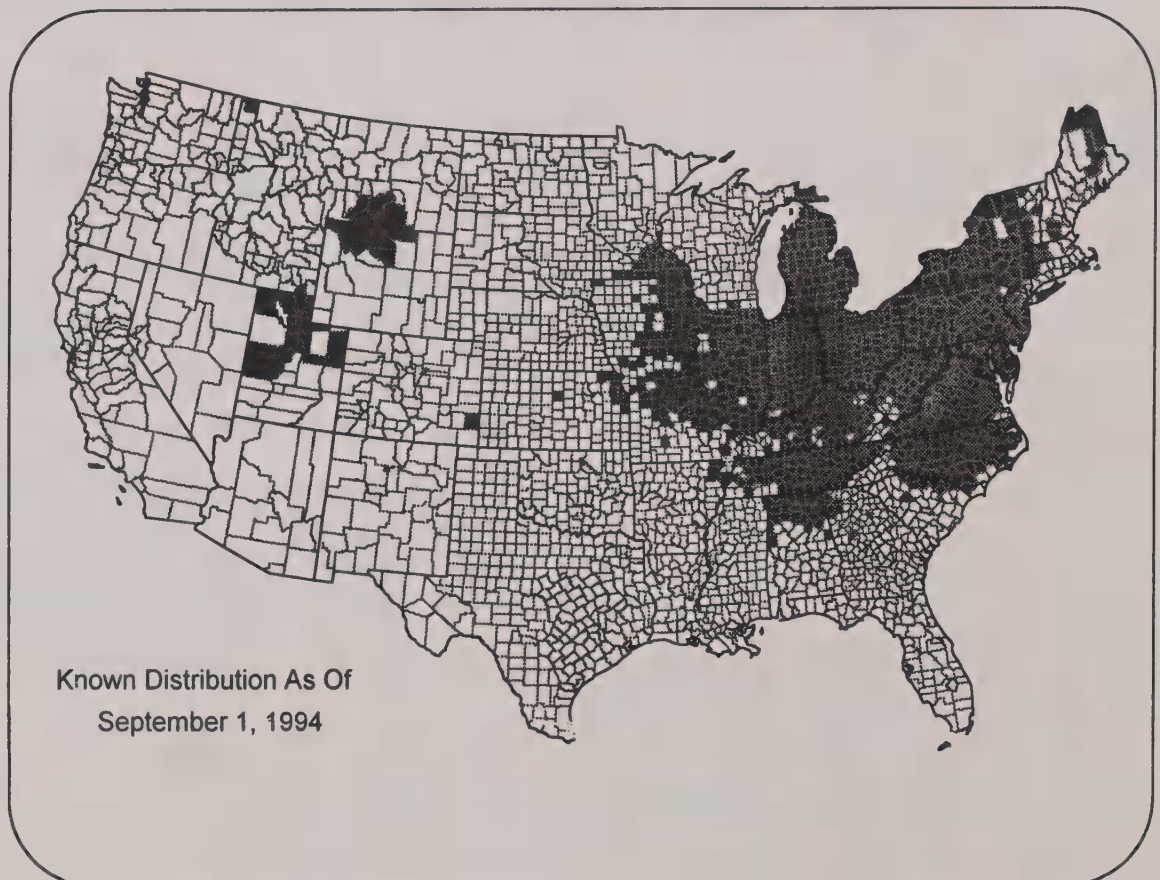


Figure 1: Known distribution range of cereal leaf beetle

INTRODUCTION

Roles and Responsibilities

Cooperators	<p>PPQ line personnel, State departments of agriculture personnel, university researchers, and Extension Service personnel will provide the technical assistance in the field to do the following tasks:</p> <ol style="list-style-type: none">1. Collect CLB eggs, larvae, and adults.2. Evaluate the establishment of natural enemies.3. Establish, maintain, and monitor field insectaries.4. Release natural enemies.
State Project Coordinators	<p>State project coordinators are generally State plant health directors (SPHD's) or officers-in-charge (OIC's) at designated PPQ locations. They are responsible for the following tasks:</p> <ol style="list-style-type: none">1. Assign cooperators designated work areas.2. Plan and coordinate work hours needed for cooperators to accomplish the tasks listed above.3. Evaluate information gathered by cooperators to ensure accuracy and completeness.4. Communicate with the Project Leader about the project's progress.
Project Leader	<p>Michael Bryan USDA, APHIS, PPQ National Biological Control Laboratory 2534 South 11th Street Niles, MI 49120 Commercial: (616) 683-3563 FAX: (616) 683-9608 E-Mail: !a348BCNILES</p>

INTRODUCTION

How to Use This Manual

Use the CLB Project Manual as an on-the-job reference when collecting CLB eggs, larvae, or adults, evaluating the establishment of natural enemies, establishing, maintaining, or monitoring field insectaries, and releasing natural enemies.

Each tabbed section is independent, containing step-by-step procedures.

Each section has an introduction which contains general information relating to the section's main content.

The Overview is a list of steps described in the section. If you are familiar with the steps, you can use the Overview as a checklist.

Use the Appendixes as they relate to the other sections of the manual. In some places an Appendix is referenced; in other places it is assumed that you accessed an Appendix to get the necessary information.

If the Contents section is not specific enough, use the Index to find a topic and its page number.

FALL COLLECTION OF CEREAL LEAF BEETLE ADULTS

Introduction

Time of Sampling

Sample in the fall, after the first frost has occurred.

Purpose

The purpose of this activity is to collect a minimum of 6,000 adult CLB's from each sampling area. For help in identifying CLB adults, see Appendix 1. Once collected, you will send the adult beetles to the National Biological Control Laboratory (NBCL), Niles, MI. NBCL will temporarily store the adult CLB's, then use them to produce thousands of eggs. These eggs will in turn be parasitized by adult female *Anaphes flavipes* that emerge from parasitized eggs collected in the field. NBCL will then ship these laboratory-parasitized eggs to release locations where the parasites will emerge and attack CLB eggs in the field.

Overview of Tasks

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for collecting adult CLB's.

1. Define collection area with State cooperators.
2. Sample fields in collection area.
3. Collect CLB adults.
4. Process collected material.
5. Pack and ship CLB adults.

FALL COLLECTION OF CEREAL LEAF BEETLE ADULTS

Define Collection Area With State Cooperators

- Step 1** Within each State identified, APHIS personnel and cooperators will identify a general area for collection of CLB adults. You can initially identify areas of infestation based on past history of CLB activity in the State.
- Step 2** Once you select a general area, locate candidate fields of wheat, oats, or barley. Fields in your collection area should have woodlots, brushy areas, or heavy fence row vegetation on the downwind side of the field. You may have to consult local weather service officials to determine the direction of prevailing winds during the fall of the year. The reason for looking for this type of field is to help you find large numbers of CLB adults (see Photographs [1-1] and [1-2] in Appendix 1). The better spots to collect are areas where the insects are concentrated due to the physical attributes of the field.
- For example, in southwest Michigan the prevailing winds come from the south and west. When adult CLB's are actively flying in the fall, they get caught up by the wind. When the wind encounters a physical feature like woods or heavy brush, the wind velocity drops, and so do the CLB's. Past experience has shown that these adult beetles enter hibernation sites at the edge of this windbreak area.
- Criteria for fields include the following:
- Oat, wheat, or barley stubble left in the field
 - Woodlots or heavy brush on downwind side(s) of the field
 - History of CLB in the area
- Step 3** Identify at least 10 candidate fields in the areas where CLB infestations are known to occur. You can identify these fields during the late summer or fall months prior to collection.

**FALL COLLECTION OF CEREAL LEAF BEETLE ADULTS
Sample Fields in Collection Area**

- Step 1** Before sampling, contact the landowner to explain the purpose, and obtain permission to sample.
- Step 2** Begin sampling after the first frost occurs. Sample when the temperature is below 65°F. You should also use prior knowledge of the appearance of CLB adults. in fields as a guideline for determining when to start sampling the fields.
- Step 3** In each field, sample along the edges of the field leeward (downwind) of prevailing winds.
- Step 4** Take 10 stems from the outside row in the field nearest the downwind side of the woodlot/brushy area. Count the number of beetles inside the stem of straw. Take stem counts every 20 paces, not to exceed 6 subsamples per field, where the bordering protection occurs. You can detect CLB's in these stems by pulling the stem between your thumb and finger.
- Step 5** Record information on sampling results so you can determine which fields have the highest density populations of CLB.

**FALL COLLECTION OF CEREAL LEAF BEETLE ADULTS
Collect Cereal Leaf Beetle Adults**

- Step 1** Determine which locations have a higher density of CLB by reviewing data from the 10 stem count. Once you find a suitable population of CLB adults, contact the landowner again to explain the collection process. Collection activities may involve a crew of three to four people working in the field for several days.
- Step 2** Assemble your equipment (cloth or paper bags, closures for the bags, rakes, and a means of cutting off the stubble such as grass shears or a hand scythe).
- Step 3** Once in the collection field, cut off all standing stubble in the outside six rows in the sheltered area previously described.
- Step 4** Place the cut stubble in the bags. When full, tie shut or otherwise close up the bags. It may be necessary to lift, using the rake, the stubble that is lying down; then cut it off. Once you have placed all the collectable material in bags, take the bags to a warm place and hang them up.

FALL COLLECTION OF CEREAL LEAF BEETLE ADULTS
Process Collected Material

- Step 1** Hold the bags containing the cut stubble in a warm place for at least 2 hours or until the next day. Before removing the adult CLB's from the bag, place several plastic storage cartons on the work surface and remove their lids. These cartons should contain rolls of corrugated cardboard.
- Step 2** Carefully open the first bag, creating a small 3-4" opening. Then holding this opening in place, turn the bag upside down, and shake the adult CLB's into the open plastic cartons.
- Step 3** Repeat until all bags have been processed.
- Step 4** Place lids on cartons and then put the cartons in the refrigerator at 45-50°F until you can ship them to NBCL.

**FALL COLLECTION OF CEREAL LEAF BEETLE ADULTS
Pack and Ship Cereal Leaf Beetle Adults**

- Step 1** After you screen and place the beetles in cartons, ship them to NBCL. You may ship beetles daily except on Saturdays and Sundays. Hold screened beetles in refrigerated storage at 40-45°F until you are able to ship them.
- Step 2** Before shipping, make arrangements with Federal Express (or the delivery service currently under Government contract) to pick up the shipment. Also, make provisions for weighing the package(s). Federal Express will need to know the weight so the account can be charged for that shipment. You may also use overnight mail.
- Step 3** Assemble your equipment and supplies. Those include the following:
- Insulated freeze safes
 - Frozen blue travel ice packs
 - Newspaper for cushioning material
 - Standard shipping labels and interstate shipping permit
 - Federal Express shipping label or overnight mail label
- Step 4** Individually wrap ice packs in four or five sheets of newspaper. You will need eight packs for the freeze safe. Each freeze safe can easily accommodate up to 50 of the plastic cartons, plus the ice packs and cushioning.
- Step 5** Place four wrapped packs on the bottom of the freeze safe. Place a layer of approximately five sheets of newspaper on top of the packs.
- Step 6** Place cartons on top of the bottom layer of ice packs.
- Step 7** Place four more ice packs on top of cartons. Fill the remainder of space with newspaper cushioning.
- Step 8** Place the lid on the freeze safe. Make sure the standard shipping label and interstate shipping permit are affixed to the freeze safe. Attach the Federal Express shipping or overnight mail label to the freeze safe. Hold the packed freeze safe in a cool area until the pickup is made.
- Step 9** Make sure you contact NBCL regarding the day and method of shipment.
- Step 10** As you approach the 6,000 mark for shipped adult CLB's, contact NBCL for further instructions regarding finishing of this phase of the collection activity.

SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS

Introduction

Time of Sampling

Sample in late winter/early spring.

Purpose

The purposes of this activity is to collect a minimum of 6,000 adult CLB's as early as possible from each sampling area. For help in identifying CLB adults, see Appendix 1. Once collected, you will send the adult beetles to the National Biological Control Laboratory (NBCL), Niles, MI. NBCL will temporarily store the adult CLB's, then use them to produce thousands of eggs. These eggs will in turn be parasitized by adult female *Anaphes flavipes* that emerge from parasitized eggs collected in the field. NBCL will then ship these laboratory-parasitized eggs to release locations where the parasites will emerge and attack CLB eggs in the field.

Overview of Tasks

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for collecting adult CLB's.

1. Define collection area with State cooperators.
2. Sample fields in collection area.
3. Collect CLB adults.
4. Temporarily store collected material.
5. Screen beetles.
6. Pack and ship CLB adults.



SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS
Define Collection Area With State Cooperators

- Step 1** Within each State identified, APHIS personnel and cooperators will identify a general area for collection of CLB adults (see Photographs [1-1] and [1-2] in Appendix 1). You can initially identify areas of infestation based on past history of CLB activity in the State.
- Step 2** Once you select a general area, locate candidate fields of wheat, oats, or barley. The best fields are those bordered by woodlots or fence rows with heavy vegetation and residues.
- Step 3** Identify at least 10 candidate fields in the areas where CLB infestations are known to occur. You can identify these fields during the late summer or fall months prior to collection.

SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS

Sample Fields in Collection Area

- Step 1** Your local climate will determine when to begin your sampling. Use your prior knowledge of the appearance of CLB adults in fields as a guideline for determining when to start sampling the fields. In North Carolina fields, collect the first samples in late February. In Virginia, start sampling 1-2 weeks later, depending on local environmental conditions. A good guideline is to sample when temperatures exceed 58°F for 6-8 hours, and night temperatures do not fall below 35°F for 2-3 consecutive nights. Small grains planted in the fall will be 6 inches or more in height at this time.
- Step 2** Before sampling, contact the landowner to explain the purpose and obtain permission to sample.
- Step 3** In each field, sample along the edges of the field leeward (downwind) of prevailing winds. You will find adult CLB's there first, after they move out of the over-wintering sites. Fields with light, sandy soils tend to have beetles before other fields.
- Step 4** Obtain the following equipment to sample for adult CLB's from the NBCL staff:
- Standard 15" diameter sweep net with spare net bags
 - Pre-Collection Data Sheet and pencil
 - White photo tray
- Step 5** In each field make 100 sweeps with a standard 15" diameter sweep net. Make sure the field is dry before using the sweep net. Since adult beetles get caught in moisture on the plants, it is best not to sweep early in the morning or after a rain. Sweep the top one-third of the plants while walking along the edges of the field. Dump the net contents into the white photo tray, and examine for presence of adult CLB's. Sweep fields when the temperature exceeds 55°F and wind speed is 0-5 mph.
- Step 6** Record the following information on the Pre-Collection Data Sheet (see a sample on the next page):
- Date
 - Field location
 - Crop
 - Number of CLB adults found
 - Surveyor's name
- When you find the first adult CLB's, contact NBCL. **Start collection immediately!**
- Step 7** Forward sampling information to the designated survey coordinator after sampling all the candidate fields.
- Step 8** Sample the 10+ candidate fields daily until you find suitable populations of adult CLB's. Selection of the collection field will be decided mutually by the survey coordinator and staff at NBCL.

CLB Pre-Collection Data Sheet

DATE	FIELD LOCATION	CROP	# OF CLB

Surveyor's Name _____

Figure 2: Pre-Collection Data Sheet

SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS

Collect Cereal Leaf Beetle Adults

- Step 1** Once you find a suitable population of CLB adults, contact the landowner again to explain the collection process. Collection activities may involve a crew of three to four people working in the field for several days.
- Step 2** Assemble the following equipment:
- Coolers with ice jugs
 - Sweep nets and spare nets
 - Paper bags
 - Large paper clips
- Step 3** Collect adult CLB's using sweep nets. When sweeping, alternate the areas swept in the field. This provides time for adult beetles which were knocked or frightened off the plants to climb back onto the plants.
- Step 4** Once you have made 100 sweeps, dump the contents of the sweep net into the paper bags provided in the equipment pack. Fold down the top of the bag twice, and attach paper clips provided for keeping the bag closed. Repeat dumping into the bag until 2-4 inches of debris are present at the bottom. Keep the bags in coolers in the shade until you can transport them to a temporary storage area. Be sure not to crush or flatten the bags when placing them in the cooler. **Make every effort to keep the collected material cool by using coolers and air-conditioned vehicles during transport.**

SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS
Temporarily Store Collected Material

- Step 1** Hold collected material in paper bags under refrigeration at 45-50°F (7-10°C) until screened. **Do not place wet debris in the bag, and do not store the bags for more than 24 hours.**
- Step 2** Expand the bags with material inside when you place them in the refrigerated area. **Do not crush or stuff the bags into a refrigerator!**

**SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS
Screen Beetles**

- Step 1** Once you have cooled down the collected beetles for 2 or more hours, screen this material. Cooling the beetles slows them down enough so they become easy to aspirate.
- Step 2** For screening the material, work on a table or bench. Assemble an aspirator, facial tissues, hand tally counter, and storage cartons.
- Step 3** Place a one-quarter piece of facial tissue in the aspirator tube and replace the aspirator head. Connect the aspirator to the source of vacuum. Vacuum should be no higher than 5 pounds.
- Step 4** Dump material to be sorted and screened from a bag into a white sorting tray. With the aspirator in one hand and counter in the other, aspirate and count the adult CLB's. Aspirate no more than 100 beetles in the tube before emptying it.
- Step 5** Dump aspirated CLB's into a storage container. Replace the lid after each dumping and put the container in the refrigerated holding area. Crew members may wish to use a cooler in the screening area to temporarily hold aspirated beetles. Place no more than 300 beetles in each carton.

SPRING COLLECTION OF CEREAL LEAF BEETLE ADULTS

Pack and Ship Cereal Leaf Beetle Adults

- Step 1** After you have screened the beetles and placed them in cartons, ship them to NBCL. You may ship daily except on Saturdays and Sundays. Hold screened beetles in refrigerated storage at 40-45°F until you are able to ship them. Before shipping, make arrangements with Federal Express (or the delivery service currently under Government contract) to pick up the shipment. Also make provisions for weighing the package(s). Federal Express will need to know the weight so the account can be charged for that shipment. You may also use overnight mail to ship the CLB's.
- Step 2** Assemble the following equipment for packaging and shipping:
- Insulated freeze safes
 - Frozen blue travel ice packs
 - Newspaper for cushioning material
 - Standard shipping labels and interstate shipping permit
 - Federal Express or overnight mailing label
- Step 3** Individually wrap ice packs in four to five sheets of newspaper. You will need eight packs for freeze safe. Each freeze safe can easily accommodate up to 50 of the plastic cartons, plus the ice packs and cushioning.
- Step 4** Place four wrapped packs on the bottom of the freeze safe. Place a layer of approximately five sheets of newspaper on top of the packs.
- Step 5** Place the cartons on top of the bottom layer of ice packs.
- Step 6** Place four more ice packs on top of cartons. Fill the remainder of space with newspaper cushioning.
- Step 7** Place the lid on the freeze safe. Make sure the standard shipping labels and interstate shipping permit are affixed to the freeze safe. Attach the Federal Express shipping or overnight mailing label to the freeze safe. Hold the packed freeze safe in a cool area until ready to ship.
- Step 8** As you near the 6,000 mark for shipped adult CLB's, contact NBCL for further instructions regarding finishing this phase of the collection activity.

COLLECTION OF PARASITIZED CEREAL LEAF BEETLE EGGS Introduction

Time of Sampling

Sample in mid to late spring.

Purpose

The purpose of this activity is to collect a minimum of 3,000 CLB eggs, some of which are parasitized by the egg parasite *Anaphes flavipes*. For help in identifying CLB eggs, see Appendix 1. Once collected, you will send the parasitized eggs to the National Biological Control Laboratory (NBCL), Niles, MI. NBCL will use the female *A. flavipes* that emerge from the parasitized eggs collected in the field to produce more parasitized eggs. NBCL will then ship these laboratory parasitized eggs to release locations where the parasites will emerge and attack CLB eggs in the field.

Overview of Tasks

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for collecting parasitized eggs.

1. Define collection area with State cooperators.
2. Sample for parasitized eggs.
3. Collect CLB eggs.
4. Pack and ship CLB eggs.

**COLLECTION OF PARASITIZED CEREAL LEAF BEETLE EGGS
Define Collection Area With State Cooperators**

- Step 1** Within each State identified, APHIS personnel and cooperators identify a general area for collection of CLB adults, and later, parasitized eggs. You can initially identify areas of infestation based on past history of CLB activity in the State.
- Step 2** Once you select a general area, locate candidate fields of wheat, oats, or barley planted in the fall. The best fields are bordered by woodlots or fence rows with heavy vegetation and residues.
- Step 3** Identify at least 10 candidate fields in the areas where CLB infestations are known to occur. You can identify these fields during the winter months before any insect activity begins.

COLLECTION OF PARASITIZED CEREAL LEAF BEETLE EGGS Sample for Parasitized Eggs

- Step 1** Once CLB adults migrate into grain fields they will mate and lay eggs. The egg parasite *Anaphes flavipes* will lay eggs on a portion of these eggs if the parasite is present in the field. Collection personnel sample candidate fields to monitor the number of eggs present. The best candidate fields are those where *A. flavipes* has been recovered in previous years. Monitor at least 10 fields daily until at least 3,000 CLB eggs are collected per State. The main focus of the sampling is to locate CLB eggs. It may be difficult to actually determine parasitization in the field.
- Step 2** CLB's prefer oats and barley over wheat. Look for eggs (Photograph [1-3] in Appendix 1) as the plants exhibit stem elongation. The approximate time will be when the plants are in pre-boot and boot stages. Look at the upper surface of the leaves, especially towards the basal portion of the leaf where it attaches to the stem. Bright yellow eggs indicate they are freshly laid. Look for the older, darker yellow CLB eggs.
- Step 3** You need no equipment to look for CLB eggs in the fields, but maintain a record of fields sampled, dates, and numbers of eggs found.
- Step 4** Within each field you sample, examine 10 stems in 5 different parts of the field. Examine each leaf for the presence of CLB eggs. Record the total number of eggs found in the field on the precollection data sheet. When you find the first eggs, immediately notify the survey coordinator. This person in turn will contact NBCL to discuss the status of the egg collection activities.
- Step 5** Continue sampling fields until NBCL decides to stop egg collection.

COLLECTION OF PARASITIZED CEREAL LEAF BEETLE EGGS

Collect Cereal Leaf Beetle Eggs

- Step 1** Once you have located suitable fields for collecting CLB eggs, contact the landowner again to explain the collection process. Collection activities may involve a crew of three to four people working in the field for several days.
- Step 2** You will need the following equipment to collect eggs:
- Coolers and ice jugs
 - Modified 1 pint paper cartons
 - Scissors
 - Forceps
 - White photo trays
 - Field Data Work Sheet (FDWS)
 - Sampling labels
- Step 3** Collect leaves of the grain plants which may contain CLB eggs (Photograph [1-3]). You can most easily collect leaves in the three outside rows of plants, but avoid any shaded areas. Break off a leaf at the base and then examine it for eggs. If eggs are present, place the leaf in a paper bag. If no eggs are present, discard the leaf. Continue this process until the bottom of the bag is lightly covered with a 1/4 inch layer of leaves.
- Step 4** Move to a shaded area where you can process the leaves for shipping.
- Step 5** Dump the leaves into the white photo tray. Grasping a leaf in one hand and a counter in the other, count the eggs on each of 50 leaves. Using a scissors, cut off the section of each leaf where you find CLB eggs. Leave a 1/2 inch margin above and below the eggs.
- Step 6** Place the cut leaf sections with eggs in a modified 1 pint carton. Put no more than 50 leaf sections in each carton. Record the number of eggs counted on a sample label. Place the label in the carton.
- Step 7** Replace the screen and paper ring. Moisten the small piece of sponge, squeeze out any excess water, and place it in the center of the screen. Put the petri dish cover over the sponge and slip the rubberband over the entire assembly (see *Figure 3*).
- Step 8** Place the carton in the cooler until it can be transferred to a refrigerated storage area prior to shipment. Ship daily the cartons of CLB eggs.

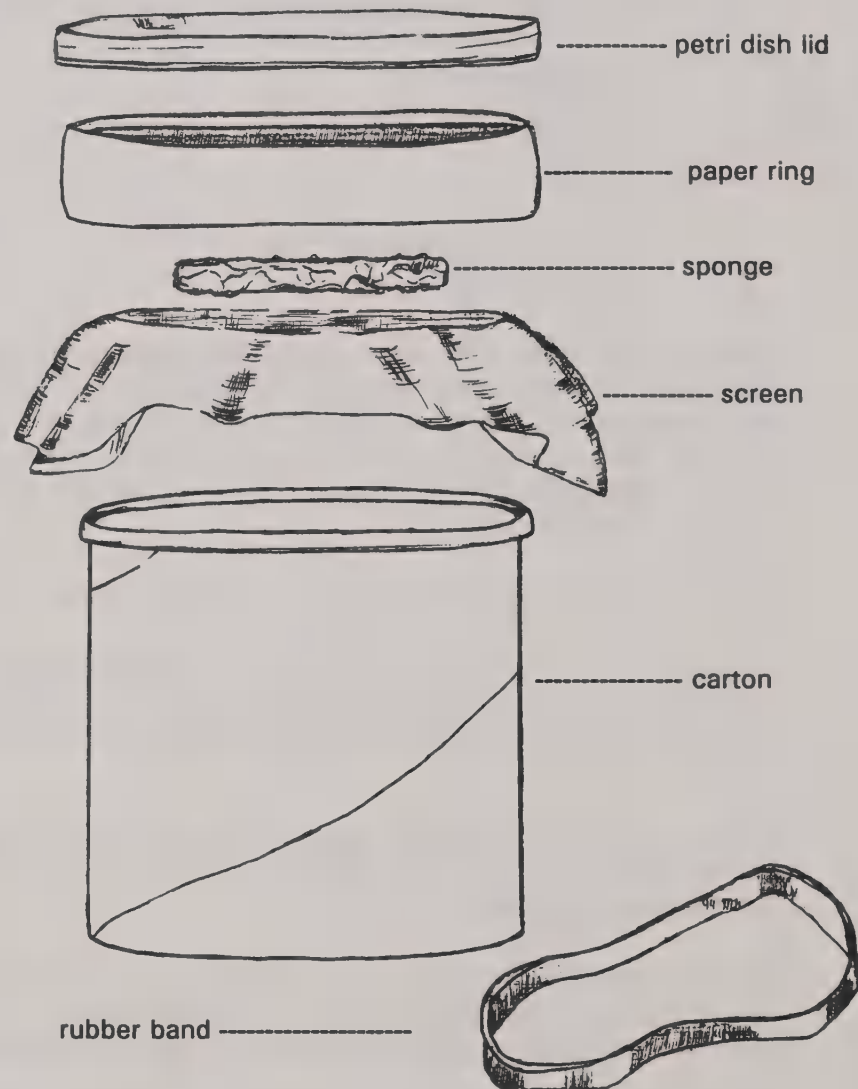


Figure 3: Collecting carton assembly

**COLLECTION OF PARASITIZED CEREAL LEAF BEETLE EGGS
Pack and Ship Cereal Leaf Beetle Eggs**

- Step 1** The process for packing and shipping cartons of CLB eggs is identical to that for shipping CLB adults. If you collect both eggs and adults at the same time, ship them in the same freeze safe.
- Step 2** As you approach the 2,500 mark of eggs shipped, contact NBCL for any further instructions regarding collection activities. If the eggs you send are not parasitized, NBCL will request additional eggs.

COLLECTION OF PARASITIZED CEREAL LEAF BEETLE LARVAE Introduction

Time of Sampling

Sample in mid to late spring.

Purpose

The purpose of this activity is to collect CLB larvae parasitized by any of the three larval parasite species *Diaparsis temporalis*, *Lemophagus curtus*, and *Tetrastichus julis* (Photographs [2-2], [2-3], and [2-4] in Appendix 2). Once collected, you will send the parasitized larvae to field insectaries for release.

Overview of Tasks

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for collecting and shipping parasitized larvae.

1. Define collection area with State cooperators.
2. Survey field for presence of CLB larvae.
3. Collect CLB larval samples.
4. Pack and ship CLB larval samples.
5. Mass collect, pack, and ship parasitized larvae.

Communication Note

The entire process of locating suitable fields, collecting, and shipping parasitized larvae requires excellent communication between the NBCL staff and field personnel. Plan to make daily arrangements to convey information about samples, amounts to be shipped, and recipient names and addresses.

Define Collection Area With State Cooperators**COLLECTION OF PARASITIZED CEREAL LEAF BEETLE LARVAE**
Define Collection Area With State Cooperators

- Step 1** Within each State identified, APHIS personnel and cooperators identify a general area for collection of parasitized CLB larvae. You can initially identify areas of infestation based on past history of CLB activity in the State.
- Step 2** Once you select a general area, locate candidate fields of oats, barley, or wheat. The best fields are bordered by woodlots or fence rows with heavy vegetation and residues.
- Step 3** Identify at least 10 candidate fields in the areas where CLB infestations are known to occur.

**COLLECTION OF PARASITIZED CEREAL LEAF BEETLE LARVAE
Survey Field for Presence of Cereal Leaf Beetle Larvae**

- Step 1** CLB's prefer oats and barley over wheat. The easiest way to locate fields containing larvae is to first find fields with eggs (Photograph [1-3] in Appendix 1) or adult beetles (Photographs [1-1] and [1-2]). Begin looking for eggs and/or adults as the plants exhibit stem elongation or tillering. The approximate time will be when winter wheat plants are in boot stage. Look at the upper surface of the leaves, especially towards the basal portion of the leaf where it attaches to the stem.
- Step 2** You need no equipment to look for CLB eggs in the fields. Simply maintain a record of the fields you survey, the dates you survey, and the amount of eggs you find.
- Step 3** After you first find CLB eggs, return to the field in 4 to 5 days to assess the progression of hatching and emergence of larvae. Once you find larvae (Photographs [1-4] and [1-5]), return every 2 to 3 days to check on larval development. Do **NOT** collect the early instar larvae; collect only third and fourth instar larvae.
- Step 4** Once you start seeing third instar larvae, send samples of the largest larvae to the Niles, Michigan National Biological Control Laboratory (NBCL) for analysis.

COLLECTION OF PARASITIZED CEREAL LEAF BEETLE LARVAE
Collect Cereal Leaf Beetle Larval Samples

- Step 1** Check to make sure you have the following items (provided by NBCL) before driving to your fields:
- Coolers and ice jugs
 - Modified 1 pint paper cartons
 - Field Data Work Sheet (FDWS)
 - Sample labels
- Step 2** In the first field, take samples of larvae (Photographs [1-4] and [1-5]) from four separate areas of the field. When you find a leaf with larvae on it, remove the leaf from the plant and place it in the modified 1 pint carton. Collect 25 large larvae from each of the 4 areas of the field.
- Step 3** Fill out a sample label for the field and place it in the carton containing the larvae. On the label include state, date, county, farmer, crop, and surveyor name. In the survey portion of the label print "collection." Print the field number (1-10) in the location section. An example is shown in *Figure 1*.
- Step 4** Replace the screen and paper ring on the carton. Moisten the small piece of sponge, squeeze out any excess water, and place it in the center of the screen. Put the petri dish cover over the sponge and slip the rubber band back over the entire assembly (see *Figure 3*).
- Step 5** Place the carton in the cooler until it can be transferred to a refrigerated storage area before shipment. Ship daily the larval samples. Do **not** collect and ship on Friday or Saturday **unless you have received special instructions to do so**.
- Step 6** Fill out a Field Data Work Sheet for the field. Complete blocks 8-29, and 32-33. An example is shown in *Figure 2*.
- Step 7** Repeat the procedure described above for other fields.
- Step 8** Remember that you are looking for high host densities with high parasitization rates.

**COLLECTION OF PARASITIZED CEREAL LEAF BEETLE LARVAE
Pack and Ship Cereal Leaf Beetle Larval Samples**

Step 1 Before packing the sample containers, freeze the travel ice packs. Also, determine where the nearest Federal Express (or the delivery service currently under Government contract) pickup location is and the deadline for receiving packages for shipment.

Step 2

If you use:	Then:
Large insulated containers	Wrap several travel ice packs in newspaper, and place in the bottom of the container.
Two-carton biomailers	Place one travel ice pack in the center compartment.

Step 3 Place modified 1 pint cartons in the insulated shipping containers, along with the respective FDWS'. If you use large insulated containers, fill excess space with crumpled newspaper.

Step 4 Close and seal the insulated shipping container. Attach an interstate shipping label and a Federal Express label.

Step 5 Take the package to the nearest Federal Express location and ship to NBCL.

Step 6 The NBCL staff will dissect the larval samples immediately after arrival. Following analysis, NBCL will contact you to relay information on the percent parasitism of the samples. You may begin collecting larvae immediately - the same day the samples are dissected at NBCL. During these discussions, the NBCL staff will relay to you species and percent parasitism rates for inclusion on a shipment FDWS. The NBCL staff will also relay any relevant shipping instructions such as recipient(s) and addresses.

COLLECTION OF PARASITIZED CEREAL LEAF BEETLE LARVAE
Mass Collect, Package, and Ship Parasitized Larvae

- Step 1** Check to make sure you have the following items (provided by NBCL) before driving to your fields:
- Large freeze safes with styrofoam insert in bottom
 - Frozen travel ice packs (8 per freeze safe)
 - Plastic containers (4 per freeze safe)
 - CLB interstate shipping labels
 - Federal Express labels
 - Return Business Reply Mailing labels
 - Cloth for plastic containers: Top (blue) and bottom (rust)
 - Rubber bands to secure top cloth to plastic container
 - Field Data Work Sheet (FDWS)
- Step 2** Before collecting the CLB larvae, prepare the plastic containers for shipment (see *Figure 4*). First, place a moistened, rust-colored cloth in the bottom of each plastic container. Second, cut off non-infested stems of the grain plants (no longer than 10 inches) and fill the container. Orient the cut portion towards the bottom. **These cut plants must be clean.** Remove all extraneous arthropods before placing the plants in the containers. Place the prepared container in the freeze safes (4 per freeze safe) and replace the freeze safe lids.
- Step 3** Transport these prepared shipping assemblies to the collection field. Also, take along the remainder of the equipment, including the frozen ice packs, top cloth, newspapers, labels, and rubberbands. At the collection field, open the freeze safes and remove the plastic containers. Replace the lids on the freeze safes.
- Step 4** Go into the field and collect large instar larvae. Remove the leaves containing large larvae and place the leaf and larvae on top of the non-infested stems in the previously prepared plastic containers. Put 600 larvae in each plastic container as illustrated in *Figure 4*.
- Step 5** Lay a dry, blue-colored cloth over the top of the container. Secure the cloth with a rubberband as indicated in *Figure 5*.
- Step 6** Put the containers of CLB larvae and plant material upright in the freeze safe. **Do not lay the containers on their sides.** Place 8 travel ice packs in the space between the containers.
- Step 7** Place the Business Reply Mailing label in a plastic bag that ziplocks secured to the inside lid of the freeze safe. Replace the lid of the freeze safe and secure the straps.
- Step 8** Move the filled and secured freeze safe to a shaded area. **Do not put the freeze safe in a closed vehicle, including its trunk.** The objective here is to keep the insects cool and alive.
- Step 9** Repeat Steps 4-8 until you are finished collecting for the day.

- Step 10** Fill out one FDWS for the collection field. Complete blocks 2-6, 8-22, 29, and 32-34. In block 3 write "600 lar./container." Place the completed FDWS in one of the freeze safes being shipped that day. If you are shipping to more than one address, complete one additional FDWS for each recipient and include in the respective shipment(s).
- Step 11** Attach a Federal Express label with the appropriate recipient address to each freeze safe. Also, attach an interstate shipping label for CLB and its natural enemies.
- Step 12** Transport the packed freeze safes to the nearest Federal Express pickup point. Ship all parasitized larvae collected that day. **Do not hold larvae overnight.**

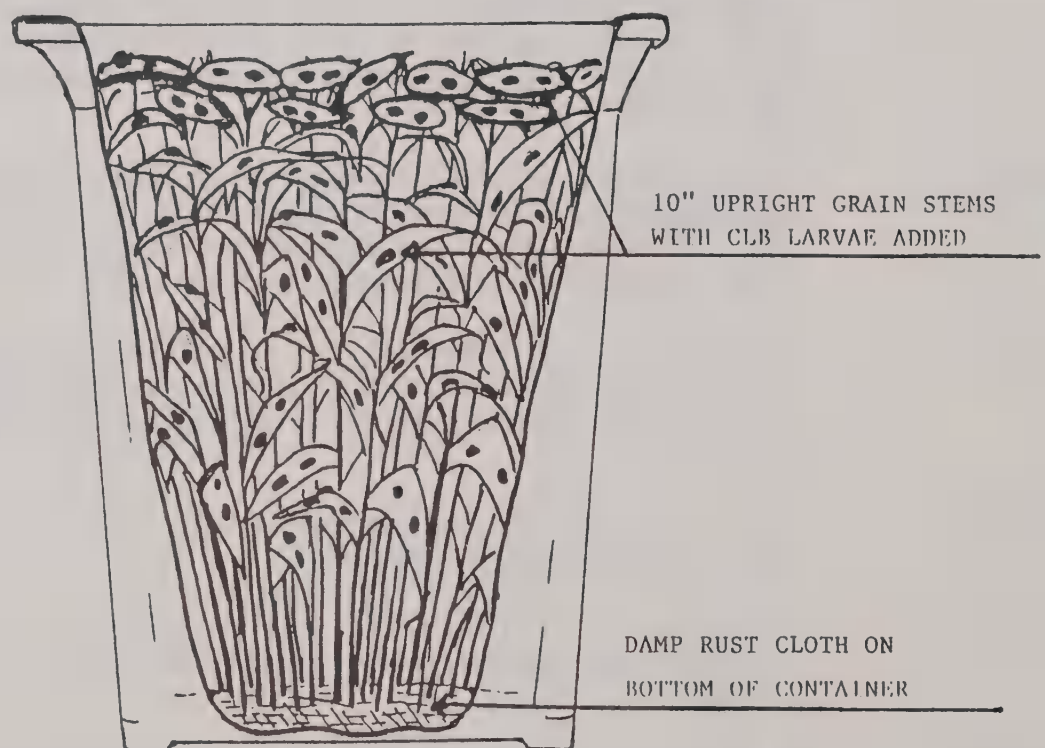


Figure 4: Cut-away showing interior of larvae transport container

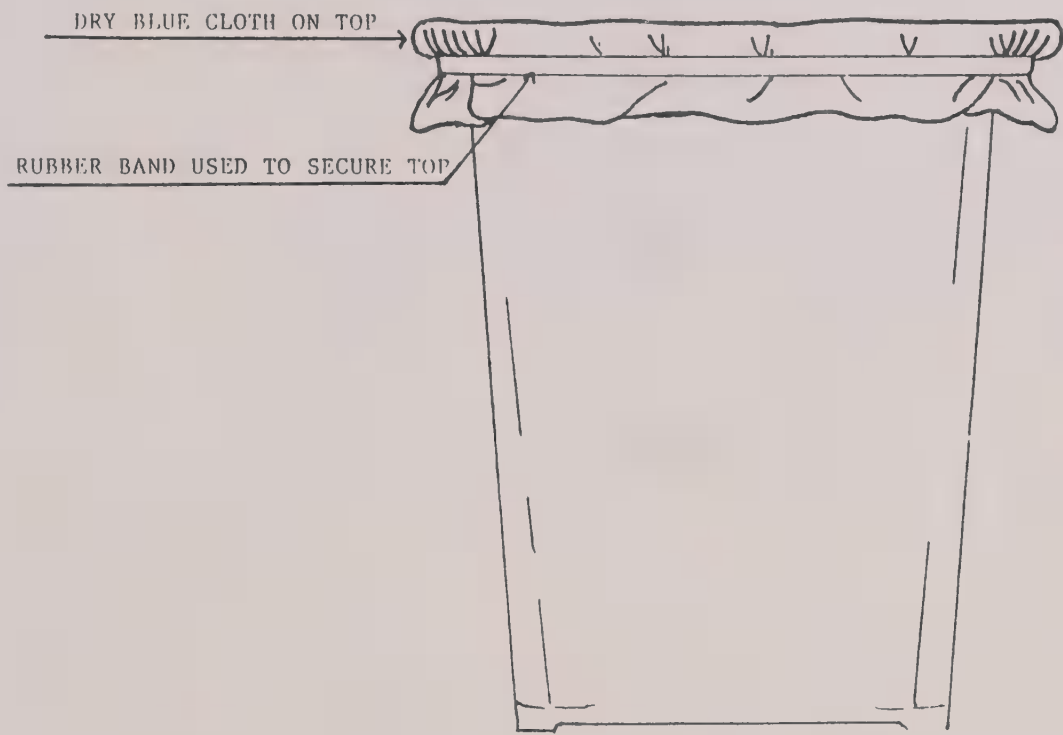


Figure 5: Covered larvae transport container

ESTABLISHMENT EVALUATION

Introduction

**Time of
Sampling**

Sample in late winter or early spring. Begin the sampling at early heading of winter wheat. Sample the fields three times. Take the second and third samples at 2 and 4 weeks respectively after taking the first sample.

Purpose

The purpose of this activity is to collect samples of CLB eggs and larvae from each of 10 fields in a sampling area. For help in identifying CLB eggs and larvae, see Appendix 1. NBCL will use these samples to determine what parasites are present in those areas sampled.

**Overview of
Tasks**

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for collecting parasitized eggs.

1. Identify sample areas.
2. Locate oat or barley fields in sampling areas.
3. Collect samples of CLB eggs.
4. Collect samples of CLB larvae.
5. Pack and ship samples.
6. Repeat sampling.

ESTABLISHMENT EVALUATION
Identify Sample Areas

- Step 1** In each State, sample one to three areas. A sampling area is roughly defined as an area 30 miles in diameter.
- Step 2** APHIS State project coordinators should contact State cooperators to acquire information regarding CLB distribution in the State.
- Step 3** In States where sampling is scheduled for more than one area, the areas should be at least 100 miles apart, if possible.
- Step 4** Contact the Project Leader at NBCL for assistance in making the final determination of sampling areas.

**ESTABLISHMENT EVALUATION
Locate Oat or Barley Fields in Sampling Areas**

- Step 1** In each sampling area, locate fields of oats or barley for sampling. If oat or barley fields are not available, substitute in order of preference spring wheat, winter wheat, or rye. If none of the above is available, call the Project Leader. Contact the Cooperative Extension Service agents as a courtesy, and to obtain more information about the location of possible fields. Do this early in the year, as farmers should know what crops they are going to plant, or have already planted.
- Step 2** Contact the landowner to explain the project and obtain permission to sample.
- Step 3** Locate and obtain permission to sample 10 fields by the time local winter wheat fields are in the boot stage. Even though winter wheat is not the crop to be sampled, it serves as a useful indicator for surveyors.
- Step 4** Sample alternate species of grasses in fence rows and along roadsides, if necessary to supplement the small grain fields.

Equipment Needed to Collect Samples:

When you collect samples, both CLB eggs and larvae may be present. Simultaneously collect both eggs and larvae (see Photographs [1-3], [1-4], and [1-5] in Appendix 1). Place egg samples (eggs on leaves) in paper cartons. Place larvae in vials containing 20 percent alcohol. Prepare for these activities by taking both types of containers to the field, along with a cooler pack and two ice jugs. Make sure you have enough paper cartons, alcohol vials, sample labels, Field Data Work Sheets (FDWS'), and related items to sample all fields designated for that day's work.

ESTABLISHMENT EVALUATION

Collect Samples of Cereal Leaf Beetle Eggs

- Step 1** In the first field to be sampled in the sampling area, examine stems and leaves of the small grain plants for the presence of CLB eggs, as well as larvae. Refer to the section on collecting larvae for details on that part of this activity. Look for CLB eggs along the upper surface of the leaf, towards the basal portion of the stem where the leaf attaches to the stem. Collect eggs from at least four separate areas throughout the field.
- Step 2** When you find eggs (Photograph [1-3]), remove the leaf from the plant. Tear off or otherwise trim off excess leaf material. Then place the leaf section with eggs in the paper carton. Keep track of the amount of leaves placed in the paper carton. Put no more than 50 leaf sections in the carton. Collect eggs from throughout the field. Do not spend more than 1 hour per sample visit per field when collecting samples of both eggs and larvae.
- Step 3** Fill out a sample label for the field and place it in the paper carton containing the eggs. On the label include State, date, county, farmer, crop, and surveyor name. In the "other" portion of the label print "Establishment" and the field number (1-10). See *Figure 6* for an example.
- Step 4** Fill out an FDWS for the field. Complete blocks 8-29, and 32-33. An example is shown in *Figure 7*.
- Step 5** Place the paper carton containing eggs and leaves in the cooler.
- Step 6** If larvae are present in the field, go to the next section, "Collect Samples of Cereal Leaf Beetle Larvae." Otherwise, move on to the next field to be sampled. After you have sampled all fields, or towards the end of the day, whichever comes first, transfer the paper cartons to the shipping box and ship to NBCL.

State	GA	Date	6-01-94
County	Hope		
Farmer	Al Cooper		
Crop	Oats		
Other	Establishment		
		Field	2
Surveyor	Ben Myers		

Figure 6: Example of a sample label

FIELD DATA WORK SHEET PLEASE PRINT				FOR LABORATORY USE ONLY			
INSTRUCTIONS: Complete original and one copy of this form. Return the original to the Biological Control Facility, APHIS, USDA, Niles, Michigan 49120. Retain the copy for your file.				1 SHP. NO.	2 DATE SHPD.	3 NO. AND STAGE SHIPPED	4 DATE COLL.
				5 SPP.	6 ORIGIN		
10 TYPE OF DATA (Check One)				7 ADJUSTED COUNTS		8 SITE DESIGNATION CODE	
<input type="checkbox"/> PRE-RELEASE SURVEY <input type="checkbox"/> COLLECTION SITE SURVEY <input type="checkbox"/> INSECTARY SITE SURVEY <input type="checkbox"/> HOST SURVEY <input type="checkbox"/> INSECTARY MONITORING <input type="checkbox"/> BIOLOGICAL ORGANISM RELEASE <input type="checkbox"/> BIOLOGICAL ORGANISM RECOVERY <input checked="" type="checkbox"/> OTHER: <u>Est. Eval.</u>				9 SPECIAL INFORMATION		GA - 2 - 1 STATE SITE FIELD SAMPLE NUMBER	
11 STATE	12 COUNTY	13 NEAREST TOWN	TOWNSHIP	29 MAP OF FIELD AND ITS LOCATION			
GA	Hopk	Ironwood		* Attached Copy of County Map. 			
14 SECTION OR OTHER DESIGNATION				15 CROP	16 NAME OF FARMER		
				oats	Al Cooper		
17 DATE DATA TAKEN				18 TIME			
5-15-94				9:30 AM			
19 CONDITIONS (Check)				20 TEMPERATURE			
<input type="checkbox"/> CLEAR <input checked="" type="checkbox"/> CLOUDY <input type="checkbox"/> CLOUDY <input type="checkbox"/> RAIN OTHER (Specify) _____				70 °			
21 WIND				22 CROP CONDITION			
FROM SW AT 5 MPH				<input checked="" type="checkbox"/> DRY <input type="checkbox"/> DAMP <input type="checkbox"/> WET <input type="checkbox"/> GOOD <input type="checkbox"/> POOR <input type="checkbox"/> MIXED STAND AGE _____ YR GROWTH STAGE: boot PLANT HEIGHT: 21 IN			
23 INSECT ADULTS				24 INSECT ADULTS			
<input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 400 SWEEPS NUMBER COLLECTED _____ NUMBER SHIPPED _____				<input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 400 SWEEPS NUMBER COLLECTED _____ NUMBER SHIPPED _____			
25 INSECT ADULTS				26 INSECT ADULTS			
<input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 400 SWEEPS NUMBER COLLECTED 100 NUMBER SHIPPED 100				<input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 400 SWEEPS NUMBER COLLECTED 50 NUMBER SHIPPED 50			
27 SURVEY COUNT METHOD				28 PESTICIDE USAGE			
<input type="checkbox"/> ACTUAL COUNT <input type="checkbox"/> 1/4 QUADRANT X 4 OTHER (SPECIFY) _____				<input type="checkbox"/> NO <input type="checkbox"/> YES DATE _____ MATERIAL _____ <input type="checkbox"/> GROUND <input type="checkbox"/> AIR			
29 SURVEYOR'S NAME, TITLE AND TEL. NO. (PLEASE PRINT)				30 DATE SUBMITTED			
Ben Myers, 712 923-709-4923				5-15-94			
31 REMARKS (e.g. SEVERE WEATHER CUTTING DATE, etc.)							

U.S. GOVERNMENT PRINTING OFFICE : 1985-6-17-543

Figure 7: Example of a Field Data Work Sheet (FDWS)

ESTABLISHMENT EVALUATION

Collect Samples of Cereal Leaf Beetle Larvae

- Step 1** In the first field to be sampled in the sampling area, examine stems of the small grain plants for the presence of large, late instar CLB larvae, as well as eggs. Refer to the section on collecting eggs for details of that part of this activity. Look for larvae along the upper surface of the leaf, towards the basal portion of the stem where the leaf attaches to the stem. Collect larvae from at least four separate areas throughout the field.
- Step 2** When you find larvae (Photographs [1-4] and [1-5]), remove the leaf containing the larvae from the plant. Then transfer the larvae from the leaf into a small scintillation vial containing 20 percent ethyl alcohol. Keep a count of the number of larvae placed in the vial. Collect larvae from at least four separate areas in the field. Collect 100 larvae from throughout the field. Place no more than 50 larvae in a vial.
- Step 3** Fill out with a pencil sample labels for the field, and place one in each vial containing the larvae. On the label include State, date, county, farmer, crop, and surveyor name. In the "other" portion of the label print "Establishment" and the field number (1-10). See *Figure 6* for an example.
- Step 4** Place the vial containing larvae in the cooler.
- Step 5** When you have finished sampling one field, move to the next field to be sampled. After you have sampled all fields, or towards the end of the day, whichever comes first, transfer the vials to the shipping carton and ship to NBCL. If you are unable to mail samples the same day you collect them, then store the samples in a refrigerator. You can store samples for 1-2 days in a refrigerator, if necessary.

**ESTABLISHMENT EVALUATION
Pack and Ship Samples**

- Step 1** Pack and ship all containers of eggs and larvae to NBCL the same day, whenever possible. Make provisions to get the boxes to the Post Office in a timely manner. Delay in the shipping of samples will result in loss of some or all of the specimens.
- Step 2** For the containers of eggs, moisten a sponge with water. Squeeze out any excess moisture. Place the sponge on the outside of the screen, then place a plastic petri dish cover over the sponge. Hold the plastic cover in place with a rubberband.
- Step 3** Place containers of eggs and/or vials of larvae in the box. Place an FDWS on top of the containers. Place no more than 5 cartons and 10 vials in one shipping box. Fill any unused space with crumpled newspaper.
- Step 4** Close and secure the lid of the box.
- Step 5** Place a mailing label on the outside of the shipping carton. Also attach an Interstate Shipping Permit, a Rush Living Insects label, and a Do Not Delay label provided in the equipment pack.
- Step 6** When you have packed all cartons, keep them cool until you are able to drop them off at the nearest Post Office.

ESTABLISHMENT EVALUATION
Repeat Sampling

Step 1 Take the second sample from each field 10-14 days after the first sample.

Step 2 Take the third sample 10-14 days after the second sample.

Adjust sampling frequency according to local conditions. For fields located in warmer climates, use a shorter period of time.

FIELD INSECTARY ESTABLISHMENT AND MAINTENANCE Introduction

Since the inception of the program for the biological control of the CLB, all attempts to mass produce larval parasites of this pest in the laboratory have been unsuccessful. Therefore, these parasites must be reared in the field. Field insectaries are an important step in the integrated CLB control program and must be selected and managed with great care.

Overview of Tasks

If you are familiar with this activity, you can use the following overview as a checklist of the tasks.

1. Select the site.
2. Choose the plot design.
3. Set up and maintain the site
4. Relocate parasitized material.

FIELD INSECTARY ESTABLISHMENT AND MAINTENANCE

Select the Site

Make sure each site you select has the following characteristics:

- Moderate to heavy recurring CLB populations
- Eight or more acres of tillable soil
- At least 5 or 6 years availability
- Rolling terrain with natural protection
- Grain crops in surrounding farm land, and
- Little possibility of insecticide drift

Land selected for use as a field insectary should contain 8-16 contiguous acres of tillable, well-drained land located in an area of moderate to heavy recurring CLB population. This land should be continuously available for a minimum of 5 or 6 years. Some possibilities that have been utilized are Agricultural Experimental Farms, State Wildlife Areas, land of State hospitals and correctional institutions, land in ASCS programs, and contracted farm land.

Locate the field insectary in an area of gently to moderately rolling terrain with scattered woodlots and heavy fence rows. Avoid flat, open areas with no protection. Land use in the surrounding areas should provide sufficient acreage of cereal grains so that the parasites can disperse easily after they have become established within the insectary.

Do not locate an insectary where there is a possibility of spring flooding or exposure to insecticide drift. Choose a site with at least a 1 mile buffer zone between the insectary and orchards, commercial truck farms, or other areas where insecticides are applied.

FIELD INSECTARY ESTABLISHMENT AND MAINTENANCE

Choose the Plot Design

When planning a field insectary, choose one of the following two types of plot design:

- Type 1 - the split-plot method (*Figure 8*). The split-plot method will yield the best results in the shortest time.
- Type 2 - the contiguous-plot method (*Figure 9*).

The field design for insectaries depends upon the terrain, amount of land available, and the direction of the prevailing spring and early summer winds. The diagrams in *Figures 8* and *9* may help in determining the best layout for your insectary. Allow a minimum of 8 acres of land in each diagram. Four acres are planted to wheat or oats each year. The remaining 4 acres are left fallow.

Design of Insectary Sites:

The insectary site split-plot design consists of a field divided into four plots (A-D). Each plot is further divided into four sections (I-IV). These plots and sections are illustrated in *Figure 8*. During each season, two of the plots will be fallow and the other two will be planted with either wheat or oats. The land use legend at the bottom of *Figure 8* shows the rotation of crops at the site. *Figure 10* shows a simplified version of a split-plot design planting design as it would appear the first year, the third and fifth years (every other year).

Insectaries using the contiguous-plot design, illustrated in *Figure 9*, consist of a field divided into two plots (A and B). As in the split plot design, one-half of the site is fallow each year. The legend at the bottom of the figure shows the rotation of crops.

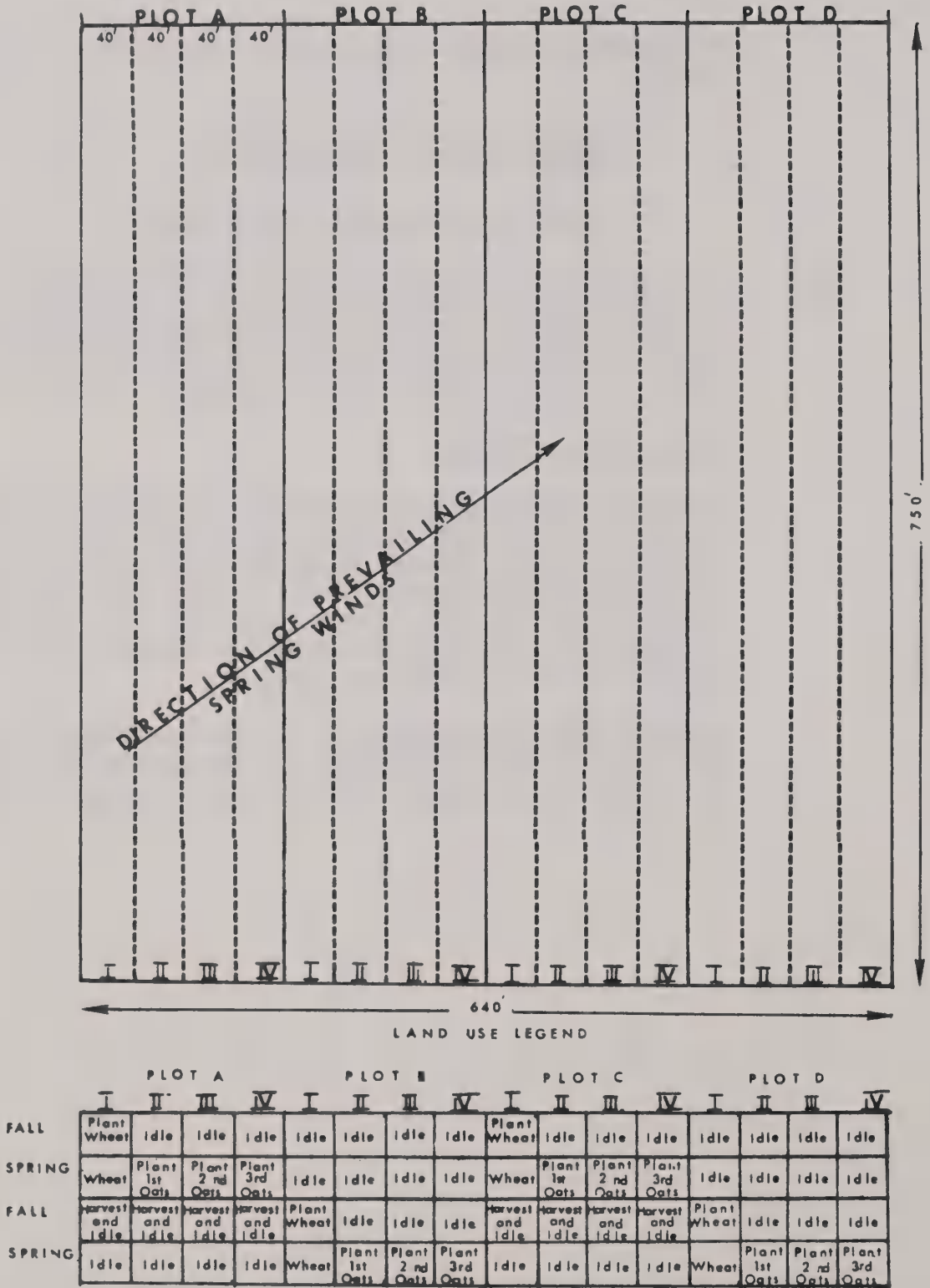


Figure 8: Example of a split plot

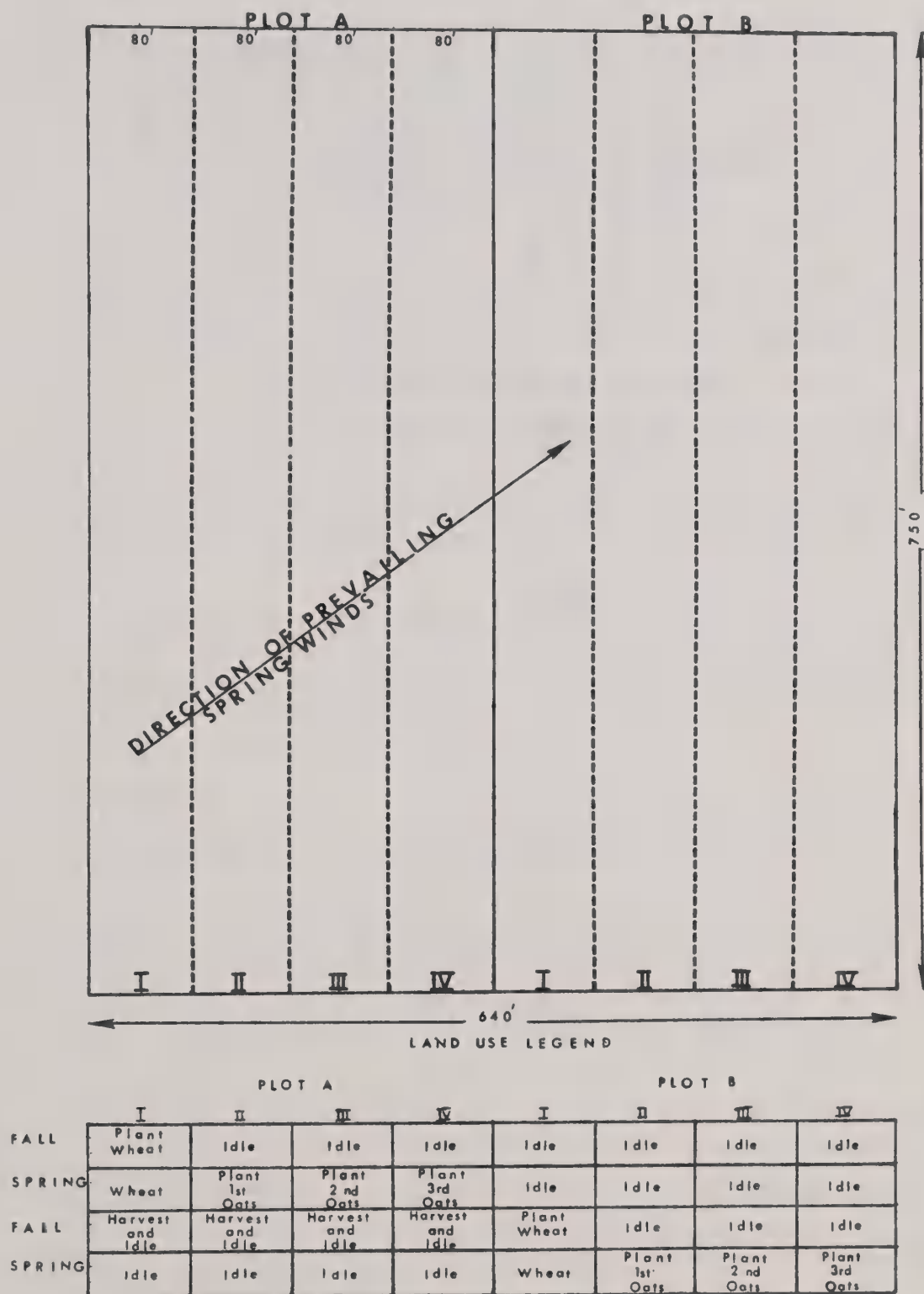


Figure 9: Example of a contiguous plot

Sec. 1	Wheat	Idle Land	Sec. 1	Wheat	Idle Land
Sec. 2	Oats		Sec. 2	Oats	
Sec. 3	Oats		Sec. 3	Oats	
Sec. 4	Oats		Sec. 4	Oats	
Plot A		Plot B	Plot C		Plot D

Figure 10: Example of a split-plot insectary

FIELD INSECTARY ESTABLISHMENT AND MAINTENANCE Set Up and Maintain the Site

Overview

Set up and maintain each site keeping the following conditions in mind:

- Direction of prevailing spring winds
- Availability of grain drill and susceptible variety of seed
- One planting of winter wheat and three plantings of oats
- Removal of crop by chopping or combining, and
- Soil surface is not to be disturbed for 2 years from planting date

During the second year, the 4 acres that were planted the first year are left undisturbed. In August or September of the second year, the section or sections that were winter wheat the first year will be plowed and planted with winter wheat in preparation for the third year insectary.

One planting of winter wheat and three plantings of spring oats should be made in each of the designated plots. Those areas designated to contain the first planting of oats are to be planted as soon as the soil can be worked in the spring, and the two successive plantings are to follow at 10 day intervals. The final planting of oats should be in the ground before the first of June in the North, and 1-2 weeks earlier than this in the South.

Two plantings of oats may be substituted if it is impossible to make the suggested three plantings. An insectary set up in this way must still follow the suggested crop sequence as shown in *Figures 8 or 9*. The only exception is that the second planting of oats would be eliminated and the late oat planting would be made approximately 20 days after the first planting.

When planning an insectary for a spring startup, it is impossible to put in the plots of winter wheat. In this case, it is advisable to double the first oat planting so space will be available for winter wheat plots in the third year.

The seed selected for planting should be a variety that has proven highly susceptible to CLB attack--Monom wheat and Clintland 64 oats were planted in the insectary in southwestern Michigan. The seed should be planted with a grain drill in well-prepared, fertile soil. Vigorous, healthy plants will attract and retain adult CLB's. This helps insure that the adult parasites have an abundance of host material to parasitize.

After the CLB larvae pupate and the crop approaches maturity, the grain should be chopped with a rotary mower, or the crop should be allowed to mature and then the grain should be harvested. The crop should not be allowed to lodge and remain on the soil surface through the fall and winter, as this will create a dense mat of vegetation. This barrier could possibly trap emerging parasites in the spring, and also delay soil warmup so that parasite emergence would no longer be in synchronization with the host.

The parasites over-winter in the top 1-2 inches of soil; therefore, it is imperative that the soil in the cropped areas not be disturbed for 2 years from planting date. **Do not** plow, cultivate, or drag the area where the parasites are over-wintering, and avoid compaction of the soil.

FIELD INSECTARY ESTABLISHMENT AND MAINTENANCE Parasite Establishment and Relocation

Before attempting to establish and build up the parasite species within an insectary, there must be sufficient, naturally occurring host population. We recommend a minimum of four larvae per square foot (1 square foot of planted grain equates to 20.5" of running row) before releasing larval parasites for establishment. It may be necessary to collect CLB in other fields and release them in the insectary to maintain the recommended host population levels.

You can measure parasite densities through insectary monitoring techniques. These techniques are outlined in another section of this manual. It is necessary to determine the rates of parasitism in the site before you can collect and relocate any material.

If high host densities are maintained and favorable weather conditions prevail, you can relocate parasitized material from the insectary during the third, fourth, and fifth years of operations.

INSECTARY MONITORING Introduction

Monitoring Visits

During the growing season you must visit the insectary to monitor the status of the crop and the CLB population. By determining when CLB adults move into the field from over-wintering sites, you can then initiate sampling for CLB eggs and larvae. Once you detect CLB eggs, you will take counts and samples of eggs and larvae. NBCL will examine the samples of eggs and larvae for parasitization by natural enemies.

Purpose

The purpose of this activity is to monitor CLB and parasite populations in the insectary. This information assures that adequate numbers of the host and natural enemies are present for collection and distribution of parasitized larvae to new areas. A minimum of 30 percent of the CLB larvae must be parasitized to support a realistic distribution program. Constant monitoring of the insectary is necessary to determine accurately this threshold. After this threshold is reached, it is essential to know which areas in the insectary contain high host densities and a high rate of parasitism. You can obtain this information by constantly monitoring each section within the insectary.

Time for Monitoring

Begin monitoring visits before winter wheat enters the boot stage. Use your prior knowledge of CLB adults in fields as a guideline for deciding when to start sampling the insectary. A good guideline is to begin monitoring after daytime temperatures exceed 58°F for 6-8 hours, and night temperatures do not fall below 35°F for 2-3 consecutive nights.

Overview of Tasks

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for insectary monitoring.

1. Assess status of CLB population.
2. Count CLB eggs and larvae.
3. Collect samples of CLB eggs.
4. Collect samples of CLB larvae.
5. Process egg samples.
6. Process larval samples.
7. Pack and ship samples.
8. Continue to take samples.

**Outline the
Monitoring
Schedule****Step 1:**

Begin monitoring prior to boot stage of winter wheat.

Step 2:

Continue monitoring at 7-10 day intervals until you find CLB larvae. Also, begin taking samples of eggs and larvae.

Step 3:

One week after you find CLB larvae, begin counting both eggs and larvae. Also, begin taking samples of eggs and larvae.

Step 4:

Take samples of eggs and larvae for a total of 4 consecutive weeks.

**Equipment for
Monitoring an
Insectary****Step 1:**

Before travelling to the insectary site, make sure you take along the following items:

- Cooler pack
- Insectary monitoring pack
- Standard sweep net

Step 2:

A minimum of 1 day before you collect egg or larval samples, fill the two, one-half gallon jugs with water and place them in the freezer. Place the frozen ice jugs in the cooler before travelling to the insectary.

Step 3:

Find the wide-mouth thermoses included in the pack. At least 24 hours before sampling for eggs, place the thermoses, with tops open, in a refrigerator.

INSECTARY MONITORING

Assess Status of Cereal Leaf Beetle Population

The purpose of assessing the status of CLB population in the insectary is to determine when to begin taking square foot counts of eggs and larvae. Begin visits when winter wheat is in the boot stage. The ideal time to start is when temperatures of 55°F or above occur for at least 3 consecutive days. This corresponds to the time when CLB adults migrate into the field. Repeat visits every 7-10 days until you find larvae. Use the following procedures during each visit:

Check for CLB Adults

Step 1:

You can easily detect CLB adults (Photographs [1-1] and [1-2] in Appendix 1) using a standard sweep net. Take 100 sweeps in one of the winter wheat sections; then examine the net contents for the adult CLB's.

Step 2:

Repeat 100 sweeps for adjacent sections of oats. Do not sweep plants that are less than 8 inches tall. If you find any adult CLB's, write "Adult CLB found" on the Field Data Work Sheet (FDWS) in block 9. Also write in the field section(s) where you found them.

Step 3:

If you found no CLB adults, write "No CLB adults found" in block 9.

Check for CLB Eggs and Larvae

Step 1:

In each section of the insectary, visually search for 5 minutes for CLB eggs, larvae, and larval feeding damage (Photographs [1-3] through [1-6]). If you find any of these, write this information in block 9 along with the section of the field. For an example of an FDWS completed at an assessment visit, see *Figure 11*.

Step 2:

Fill in blocks 9-23 and 32-34 on the FDWS.

Step 3:

Mail the white copy of the FDWS to NBCL. Mail the pink copy to your APHIS State project coordinator. Retain the yellow copy for your records.

Continue Visits

Repeat visits every 7-10 days until you locate CLB larvae. Complete an FDWS each time you visit the site. Once you find CLB larvae, stop assessment procedures, and begin taking square foot counts of eggs and larvae. For details of these procedures, go to the next section, "Count Cereal Leaf Beetle Eggs and Larvae."

FIELD DATA WORK SHEET PLEASE PRINT				FOR LABORATORY USE ONLY			
INSTRUCTIONS: Complete original and one copy of this form. Return the original to the Biological Control Facility, APHIS, USDA, Niles, Michigan 49120. Retain the copy for your file.				1. SHIP NO.	2. DATE SHIPD	3. NO. AND STAGE SHIPPED	4. DATE COLL.
10. TYPE OF DATA (Check one)				5. SPP.		6. SITE DESIGNATION CODE	
<input type="checkbox"/> PRE-RELEASE SURVEY <input type="checkbox"/> HOST SURVEY <input type="checkbox"/> BIOLOGICAL ORGANISM RECOVERY				<input type="checkbox"/> COLLECTION SITE SURVEY <input checked="" type="checkbox"/> INSECTARY MONITORING <input type="checkbox"/> OTHER: <u>Assessment</u>		<input type="checkbox"/> INSECTARY SITE SURVEY <input type="checkbox"/> BIOLOGICAL ORGANISM RELEASE	
11. STATE: <u>TN</u> 12. COUNTY: <u>Newport</u> 13. NEAREST TOWN: <u>Harrison</u>				9. SPECIAL INFORMATION			
14. SECTION OR OTHER DESIGNATION: <u>Harrison Insectary</u>				ALL eggs found larvae. 12 CLB adults LII 0 eggs larvae no adults found			
15. CROP: <u>wheat</u>				STATE: _____ SITE: _____ FIELD: _____ SAMPLE NUMBER: _____			
16. NAME OF FARMER: <u>George Zell</u> PHONE #: _____ 17. DATE DATA TAKEN: <u>5-18-91</u> 18. TIME: <u>9:30 AM</u>				29. MAP OF FIELD AND ITS LOCATION			
19. CONDITIONS (Check one) <input type="checkbox"/> CLEAR <input checked="" type="checkbox"/> PT. CLOUDY <input type="checkbox"/> CLOUDY <input type="checkbox"/> RAIN OTHER (Specify): _____				20. TEMPERATURE: <u>66° F</u> 21. WIND: FROM <u>NE</u> AT <u>5</u> MPH 22. CROP CONDITION: <input type="checkbox"/> DRY <input type="checkbox"/> DAMP <input checked="" type="checkbox"/> WET 23. CROP CONDITION: <input checked="" type="checkbox"/> GOOD <input type="checkbox"/> POOR <input type="checkbox"/> PURE <input type="checkbox"/> MIXED STAND AGE: _____ YR. GROWTH STAGE: <u>heading</u> PLANT HEIGHT: <u>2.10</u> III			
24. INSECT ADULTS: <input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 200 SWEEPS NUMBER COLLECTED: _____ NUMBER SHIPPED: _____				25. INSECT LARVAE: <input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 200 SWEEPS NUMBER COLLECTED: _____ NUMBER SHIPPED: _____ 26. OTHER ORGANISM STAGE (Specify): _____ <input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 200 SWEEPS NUMBER COLLECTED: _____ NUMBER SHIPPED: _____ 27. SURVEY COUNT METHOD: <input type="checkbox"/> ACTUAL COUNT <input type="checkbox"/> 1/4 QUADRANT x 4 <input type="checkbox"/> OTHER (SPECIFY): _____			
28. PESTICIDE USAGE: <input type="checkbox"/> NO <input type="checkbox"/> YES DATE: _____ MATERIAL: _____ <input type="checkbox"/> GROUND <input type="checkbox"/> AIR				30. CONDITION OF RELEASE MATERIAL			
33. SURVEYOR'S NAME, TITLE AND TEL. NO. (PLEASE PRINT): <u>Joe Carpenter, PPQ 801-663-0217</u>				31. NUMBER OF DEAD IN RELEASE CONTAINER (ADULT RELEASE ONLY): _____			
34. DATE SUBMITTED: <u>5-20-94</u>				32. REMARKS (e.g., SEVERE WEATHER, CUTTING DATE, etc.):			

Wooded Area

A	B	C	D
Grass	Spring oats	Early oats	Fallow
			Fall wheat

County Rd. Tiny's Pig Farm

Field Cow Pasture

U.S. GOVERNMENT PRINTING OFFICE: 1985-641-543

Figure 11: Example of a Field Data Work Sheet (FDWS) completed at an assessment visit

INSECTARY MONITORING
Count Cereal Leaf Beetle Eggs and Larvae

- Step 1** Start sampling in the first oat section next to the winter wheat planting. Lay down the 20.5" measuring stick provided along a row of plants at least 6 feet from the end of the row. The plants in this measured area equate to a square foot of standard planted field.
- Step 2** Count all CLB eggs and larvae (Photographs [1-3], [1-4], and [1-5]) in the 20.5" row. If egg numbers have dwindled to nothing, still take larval counts.
- Step 3** Repeat Steps 1 and 2 at two other areas in that section.
- Step 4** Fill in blocks 9-23 and 32-34 on the FDWS. An example of an FDWS completed while taking egg and larval counts is shown in *Figure 12*.
- Step 5** Enter the section, the average number of eggs, average larvae per square foot found in that section, and the predominant larval instar in block 9 of the FDWS.
- Step 6** Repeat Steps 1-5 for the other oat sections in the insectary. Once you have completed this, go to the next section, "Collect Samples of Cereal Leaf Beetle Eggs."

FIELD DATA WORK SHEET PLEASE PRINT				FOR LABORATORY USE ONLY			
INSTRUCTIONS: Complete original and one copy of this form. Return the original to the Biological Control Facility, APHIS, USDA, Niles, Michigan 49120. Retain the copy for your file.				1. SHP NO.	2. DATE SHPD	3. NO. AND STAGE SHIPPED	4. DATE COLL.
10. TYPE OF DATA (Check one)				5. SPP.	6. ORIGIN		
<input type="checkbox"/> PRE-RELEASE SURVEY <input type="checkbox"/> HOST SURVEY <input type="checkbox"/> BIOLOGICAL ORGANISM RECOVERY				7. ADJUSTED COUNTS		8. SITE DESIGNATION CODE	
<input type="checkbox"/> COLLECTION SITE SURVEY <input checked="" type="checkbox"/> INSECTARY MONITORING <input type="checkbox"/> OTHER _____				9. SPECIAL INFORMATION		STATE _____ SITE _____ FIELD _____ SAMPLE NUMBER _____	
11. STATE <u>TN</u> 12. COUNTY <u>Newport</u> 13. <input checked="" type="checkbox"/> NEAREST TOWN <u>Harrison</u> <input type="checkbox"/> TOWNSHIP _____ 14. SECTION OR OTHER DESIGNATION <u>Harrison Insectary</u> 15. CROP <u>Oats</u> ACRES _____ 16. NAME OF FARMER <u>George Bell</u> PHONE # _____ 17. DATE DATA TAKEN <u>6-01-94</u> TIME <u>10:00</u> (A.M./P.M.) 19. CONDITIONS (Check) <input checked="" type="checkbox"/> CLEAR <input type="checkbox"/> P1 CLOUDY <input type="checkbox"/> CLOUDY <input type="checkbox"/> RAIN OTHER (Specify) _____ 20. TEMPERATURE <u>65 F</u> 21. WIND FROM <u>Calm</u> MPH <u>0</u> 22. CROP CONDITION <input checked="" type="checkbox"/> DRY <input type="checkbox"/> DAMP <input type="checkbox"/> WET 23. CROP CONDITION <input checked="" type="checkbox"/> GOOD <input type="checkbox"/> POOR <input type="checkbox"/> PURE <input type="checkbox"/> MIXED STAND AGE _____ YR GROWTH STAGE <u>Heading</u> PLANT HEIGHT <u>22</u> IN 24. INSECT ADULTS <input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 200 SWEEPS NUMBER COLLECTED _____ NUMBER SHIPPED _____ 25. INSECT LARVAE <input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 200 SWEEPS NUMBER COLLECTED _____ NUMBER SHIPPED _____ 26. OTHER ORGANISM STAGE <input type="checkbox"/> 100 SWEEPS <input type="checkbox"/> 200 SWEEPS NUMBER COLLECTED _____ NUMBER SHIPPED _____ 27. SURVEY COUNT METHOD <input type="checkbox"/> ACTUAL COUNT <input type="checkbox"/> 1/4 QUADRANT x 4 <input type="checkbox"/> OTHER (SPECIFY) _____ 28. PESTICIDE USAGE <input type="checkbox"/> NO <input type="checkbox"/> YES DATE _____ MATERIAL _____ <input type="checkbox"/> GROUND <input type="checkbox"/> AIR 33. SURVEYOR'S NAME, TITLE AND TEL. NO. (PLEASE PRINT) <u>Joe Carpenter, PPQ 801-663-0317</u> 34. DATE SUBMITTED <u>6-01-94</u>				29. MAP OF FIELD AND ITS LOCATION 			
30. CONDITION OF RELEASE MATERIAL				31. NUMBER OF DEAD IN RELEASE CONTAINER (ADULT RELEASE ONLY)			
32. REMARKS (e.g., SEVERE WEATHER, CUTTING DATE, etc.)							

* U.S. GOVERNMENT PRINTING OFFICE : 1985-641-543

Figure 12: Example of a Field Data Work Sheet (FDWS) completed while taking egg and larval counts

INSECTARY MONITORING

Collect Samples of Cereal Leaf Beetle Eggs

While taking counts of eggs [Photographs [1-3]], you will have identified which sections of oats contain CLB eggs. These are the sections in which you will take samples of the eggs.

- Step 1** In the first section to be sampled, remove a blade of host plant containing the CLB eggs and place it in a plastic bag that zip locks. Continue this process, randomly selecting eggs from the section until you have collected 100 eggs, or until you have spent 15 minutes searching in the section.
- Step 2** After collecting eggs, fill out a sample label. Using a pencil, record the State, date, county, and crop. Record "IM" in the "Other" section of the label. Refer to *Figure 13* for an example. Place the label in a plastic bag that zip locks, seal the bag, and place it in the cooler.
- Step 3** Repeat sampling procedures in Steps 1 and 2 in the other planted sections where you found eggs during the counting portion of the visit.
- Step 4** After completing egg sampling, go to the next section, "Collect Samples of Cereal Leaf Beetle Larvae."

State TN	Date 6-1-94
County Newport	
Farmer	
Crop Oats	
Other IM	
Surveyor Joe Carpenter	

Figure 13: Example of a sampling label, after collecting larvae

INSECTARY MONITORING

Collect Samples of Cereal Leaf Beetle Larvae

While taking counts of larvae (Photographs [1-4] and [1-5]), you will have identified sections of oats containing CLB larvae. These are the sections in which you will take samples of the larvae.

- Step 1** In the first section to be sampled, remove the blade of host plant containing the CLB larvae and place it in a plastic bag that zip locks. Continue this process, randomly selecting larvae from the section until you have collected 100 larvae, or until you have spent 15 minutes searching in the section.
- Step 2** After collecting larvae, fill out a sample label. Using a pencil, record the State, date, county, and crop. Record "IM" in the "Other" section of the label. Refer to *Figure 13* for an example. Place the label in a plastic bag that zip locks, seal the bag, and place it in the cooler.
- Step 3** Repeat sampling procedures in Steps 1 and 2 in the other planted sections where you found larvae during the counting portion of the visit.
- Step 4** Keep the plastic bag that zip locks containing the CLB larvae cool until you are able to process them.
- Step 5** After completing larval sampling, go to the sections relating to processing samples.

INSECTARY MONITORING

Process Egg Samples

- Step 1** Move to a shaded area where you can process the leaves for shipping. You may wish to do this indoors. Process one subsample at a time.
- Step 2** Remove a paper placement grid from the equipment pack. See *Figure 14* for an example. Place a small amount of petroleum jelly on the grid. Remove a glass cover slip from its box in the equipment pack. Place the cover slip on the grid. The petroleum jelly acts as an adhesive, holding the cover slip to the grid.
- Step 3** Dump leaves from the first subsample into the white photo tray.
- Step 4** Pick out a leaf and uncurl it gently with the probe. Hold the leaf down at both ends with your fingers. Gently remove eggs using the side of the probe and place them on the cover slip. Place the eggs at the intersections of the lines. Repeat for any other eggs on the leaf. The eggs have a sticky substance at the point of attachment to the leaf that makes them stick to the leaf.
- Step 5** Repeat Step 4 until all leaves in the subsample are processed. Place eggs in rows of 10, and place no more than 100 eggs on a cover slip.
- Step 6** Remove and open a petri dish from the equipment pack. Inside the bottom of the dish, place a small amount of petroleum jelly in the center. Remove a filter paper from the box in the equipment pack and place it on top of the petroleum jelly. Add a small amount of the petroleum jelly to the center of the filter paper. Gently press down, causing the filter paper to adhere to the petri dish. Carefully remove the cover slip from the grid and place it on top of the filter paper. Using the dropper vial provided in the equipment pack, place two drops of distilled water on the filter paper. Avoid getting water drops on the cover slip. Replace the lid.
- Step 7** Using a felt tip laundry marker provided in the equipment pack, label the petri dish lid with the date, insectary location, section, and number of eggs.
- Step 8** Place a crumpled facial tissue in the bottom of a **chilled** wide-mouth thermos. Lay the petri dish on top of the tissue.
- Step 9** Repeat this process for all egg samples. Place no more than five petri dishes in a thermos. Place facial tissue(s) in the thermos to fill any voids and to cushion petri dishes during shipment. After processing all subsamples, go to the section "Pack and Ship Samples."

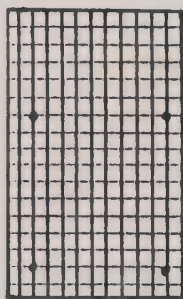


Figure 9: Example of a paper placement grid from the equipment pack

**INSECTARY MONITORING
Process Larval Samples**

- Step 1** Move to a shaded area where you can process the leaves for shipping. You may wish to do this indoors. Process one subsample at a time.
- Step 2** Remove and open a small vial from the equipment pack. Place a small amount of 20 percent alcohol in the vial.
- Step 3** Dump all the leaves from the first subsample into the white photo tray. Remove the sample label from the subsample, and place it in the vial.
- Step 4** Pick up a leaf. Using the side of the forceps, gently push any larvae into the vial. Do not squeeze the larvae with the forceps. Repeat for any other larvae on the leaf.
- Step 5** Repeat Step 4 until you have processed all leaves in the subsample and all larvae from the subsample in the vial.
- Step 6** Replace the vial cap securely so that the vial does not leak.
- Step 7** Repeat this process for all larval samples.
- Step 8** When you have processed all subsamples, go to the section entitled "Pack and Ship Samples."

**INSECTARY MONITORING
Pack and Ship Samples**

- Step 1** Pack and ship to NBCL all samples of eggs and larvae the same day you collect them. Ship the cartons by First Class Business Reply Mail. Make provisions to get the box to the Post Office in a timely manner. Delays in shipping samples will result in loss of some or all of the egg specimens.
- Step 2** Open the small cardboard shipping box. Place crumpled newspaper in the box for cushioning.
- Step 3** Place any wide-mouth thermoses containing egg samples in the box.
- Step 4** Place all vials of larval samples in a plastic bag that zip locks. Seal the bag, and place it in the box. Place more crumpled newspaper in the box to secure the samples.
- Step 5** Place the completed white copy of the FDWS in the box. Mail the pink copy to your APHIS State project coordinator, and retain the yellow copy for your records. Close the box and seal with packing tape.
- Step 6** Place a First Class Business Reply Mail label on the outside of the box. Also attach an Interstate Shipping Permit, a Rush Living Insects Label, and a Do Not Delay label provided in the equipment pack.
- Step 7** Keep the boxes cool before dropping them off at the nearest Post Office.

INSECTARY MONITORING
Continue to Take Samples

After initially collecting larval samples, continue to take eggs and larvae for another 3 weeks. After collecting larval samples for a total of 4 weeks, stop the sampling for the rest of the growing season.

RELEASES OF NATURAL ENEMIES

Introduction

**Time of
Releases**

Release in the spring months.

Purpose

The purpose of this activity is to release and establish natural enemies in fields containing CLB populations. Synchronize releases with the occurrence of the appropriate CLB life stage. In other words, release egg parasites when CLB eggs are present in the field; release larval parasites when larvae are present.

**Overview of
Tasks**

If you are familiar with this activity, you can use the following overview as a checklist of the tasks for releasing natural enemies.

1. Locate candidate release fields.
2. Sample for CLB eggs.
3. Release the egg parasite *Anaphes flavipes*.
4. Perform followup release maintenance activities.
5. Release larval parasites in the adult form.
6. Release larval parasites as parasitized larvae.

RELEASES OF NATURAL ENEMIES
Locate Candidate Release Fields

- Step 1** Determine areas in the State for release. Contact the Project Leader at NBCL, Niles, MI, for details on the release area.
- Step 2** Within the release area locate at least 10 fields of wheat, oats, or barley where there is a history of CLB populations. You can locate these fields through contacts with the local Extension Service.
- Step 3** Before sampling, contact the landowner/grower for permission to sample. Explain the purpose of the sampling and the project.
- Step 4** Make sure that the release field meets the following criteria: (1) No pesticides should be used, and (2) Other grain fields should be in the vicinity for that year and following years. In addition, the ideal release field should not be tilled until the following year to permit successful over-wintering of the parasite. Whenever possible, select fields where the grower is using the grain as a nurse crop for a pasture or hay crop.
- Step 5** Continue contacting growers until you have located 10 grain fields that meet at least criteria (1) and (2) in Step 4.

RELEASES OF NATURAL ENEMIES
Sample for Cereal Leaf Beetle Eggs

- Step 1** Begin sampling when winter wheat is in the stages of stem elongation to pre-boot.
- Step 2** In the first candidate field, locate a sample area at random. Simply toss the 20.5" measuring stick into the field. Sample in the row where it lands. Lay the stick along the edge of the row.
- Step 3** Examine the plants in this 20.5" section for presence of CLB eggs. Count all eggs found. Record this information on the FDWS in block 9.
- Step 4** Repeat counts in three other areas of the field.
- Step 5** Fill out blocks 10-23 and 28-34 on the FDWS.
- Step 6** Repeat steps 2-5 for the other candidate fields in the area.
- Step 7** Mail the white copy of the FDWS to NBCL, Niles, MI. Retain the yellow copy for your records, and forward the pink copy to your State project coordinator.
- Step 8** Repeat sampling at weekly intervals until you find an average of two or more CLB eggs per square foot in any field. When you find eggs at this density or higher, the State project coordinator will contact NBCL to arrange for shipment of the egg parasite *Anaphes flavipes*. The State project coordinator and the CLB Project Leader at NBCL will select the best field out of the candidates for release.



RELEASES OF NATURAL ENEMIES

Release the Egg Parasite *Anaphes flavipes*

Anaphes flavipes (Photograph [2-1] in Appendix 2) attacks newly-laid CLB eggs. This parasite may have five or more generations per year on CLB eggs in host crops.

- Step 1** Hold eggs between 40-50°F until you take them to the field for release. If held at room temperature, adult parasites will emerge within 24-48 hours. **DO NOT KEEP EGGS IN THE TRUNK OR INSIDE A CLOSED VEHICLE ON A WARM DAY!**
- Step 2** Host eggs parasitized by *A. flavipes* will be shipped to you inside small petri dishes. These in turn will be shipped in insulated containers. One or more 1-pint milk cartons will be sent along with the shipment. Each carton serves as a container for housing one to two petri dishes in the field. The carton protects the parasitized eggs from weather and predators until the adult parasites emerge. After emerging, the adult parasites will disperse into the field from the carton.
- Step 3** Tape the carton (holes down) to a clump of grass or host crop (see *Figure 15*) on the edge of or in the release field. It may be necessary to use tall stakes or posts if the vegetation is not tall enough to use as a support. Shipments of *A. flavipes* will contain enough tape to attach the cartons to the upright material.
- Step 4** Remove a piece of sponge from the shipping carton. Moisten with water. Squeeze to remove excess water. Open the carton and place the piece of moistened sponge on what is now the bottom of the carton. Remove petri dishes from the insulated wide-mouth thermos.
- Step 5** On the lid of each petri dish will be a code number. Record the code number(s) in block 9 of the FDWS.
- Step 6** Remove the lid of the petri dish containing the parasitized eggs. Place no more than two such lids on top of the moistened sponge with the egg side up. Place the bottoms back in the wide-mouth thermos. Ship the bottoms back to NBCL, Niles, MI, the following week, along with the spent eggs and petri dish tops.
- Step 7** Close the flap on the carton, and secure with the binder clips provided.
- Step 8** Fill out blocks 10-23 and 28-34 on the FDWS. Draw a map of the release field and surrounding landmarks in block 29 of the FDWS. On the map, also indicate approximate release points in the field. If you released *A. flavipes* in this field earlier in the year and have completed an FDWS with a map, it is not necessary to draw another map with this field. Return the white copy of the FDWS to NBCL. Retain the yellow copy for your records, and forward the pink copy to your State project coordinator.
- Step 9** If you have scheduled releases for more than one field in an area, the fields must be at least 6 miles apart.



Figure 15: Drawing of Anaphes flavipes release carton depicting proper mounting procedure

RELEASES OF NATURAL ENEMIES

Followup Release

- Step 1** After you have set up *Anaphes flavipes* release cartons in the release field, you must return to the site to check the sponge's moisture in the carton. The moist sponge keeps the humidity level high enough so the parasitized eggs do not dry out. If the eggs become too dry, the adult parasites inside cannot emerge.
- Step 2** Return to the field the second and fourth day after release to make sure the sponge is moist in each release carton. If the weather is hot and dry, you may need to check the sponges every day for 5 days.
- Step 3** Once you are in the release field, remove the binder clips of each release carton that you placed in the field. Carefully open up the carton. Remove the sponge and soak with water. Squeeze out any excess water; then return the sponge to the carton. Close up the lid of the carton, and secure it with clips.
- Step 4** One week after setting out the parasitized eggs, return to the field to remove the petri dishes from the carton.
- Step 5** Open up each release carton set out the previous week. Remove the petri dish lids from each carton. Place a bottom on each lid. Place the used petri dishes back into the wide-mouth thermoses in which they were originally shipped.
- Step 6** Place the wide-mouth thermos(es) in the mailing container provided. Mail these back to NBCL, Niles, MI, for quality control evaluation of the release stock.
- Step 7** After you have made the last release, and when the last of the CLB eggs are laid, take a sample of eggs. Take this sample when you find fourth instar CLB larvae.
- Step 8** Collect these samples at least 5 feet from any release carton, but no more than 50 feet away. Examine plants for CLB eggs. Randomly select leaves containing eggs. Place the leaves in a white 1-pint paper carton previously provided in one of your release shipments. Also place a dampened piece of paper towel in the carton to maintain high humidity levels. Collect a sample of 100 eggs in this manner.
- Step 9** Place the white carton with leaf samples in the mailing container provided. Mail these back to NBCL for evaluation of the establishment success.

RELEASES OF NATURAL ENEMIES

Release Larval Parasites in the Adult Form

General Information

Diaparsis temporalis (Photograph [2-2]) - attacks larval stages, particularly second and third instars; one generation per year; solitary parasite (one parasite per host).

Lemophagus curtus (Photograph [2-3]) - attacks larval stages, particularly second and third instars; commonly has two generations per year; solitary parasite (one parasite per host).

Tetrastichus julis (Photograph [2-4]) - attacks larval stages, particularly second and third instars; commonly has two generations per year; gregarious parasite species (more than one produced per host larvae).

Step 1

When releasing CLB larval parasites, whether as adults or as parasitized larvae, it is essential that the release field not be plowed or otherwise tilled until after July 1 the following year. The CLB larval parasites remain in the soil from mid-summer until late the following spring. If the release site is tilled during this time, the parasites will be destroyed.

Step 2

The parasite adults are shipped in pint cartons. Release these species by allowing adults to escape from open cartons under the plant canopy. Release adults at several locations within a field. You can do this by allowing a few parasites to escape from the carton, then moving the carton to another area within the field.

Step 3

Release parasite adults when you can find at least one CLB larva per 10 plant stems (4 larvae per 20.5" linear row). New infestations require only two larvae per square foot. Adults live from 2-4 weeks.

Step 4

If necessary (for example, during inclement weather), you may hold parasite adults at 45-60°F before releasing them. **DO NOT KEEP RELEASE MATERIAL IN THE TRUNK OR INSIDE A CLOSED VEHICLE ON A WARM DAY!** Provide honey and water every 24 hours in the following manner:

Cover the end of the container with a piece of screen. Streak three lines of honey on the screen with a toothpick or single-hair brush. Place the carton on its side. Using the dropper bottle, place two to three drops of water next to the top end of each honey streak. Allow the water to run down the screen. Avoid getting water inside the carton. Turn the open end toward the light and allow parasites to feed and exercise for 30 to 60 minutes. Store at the prescribed temperature until you can release the parasites.

Step 5

Write the code number from each carton in block 9 of the FDWS.

Step 6

Fill out blocks 10-23 and 28-34 on the FDWS. Draw a map of the release field and surrounding landmarks in block 29 of the FDWS. On this map, also indicate approximate release points in the field.

Step 7

Return the white copy of the FDWS to NBCL, Niles, MI, along with the release cartons and shipping container. Retain the yellow copy for your records, and forward the pink copy to your State project coordinator.



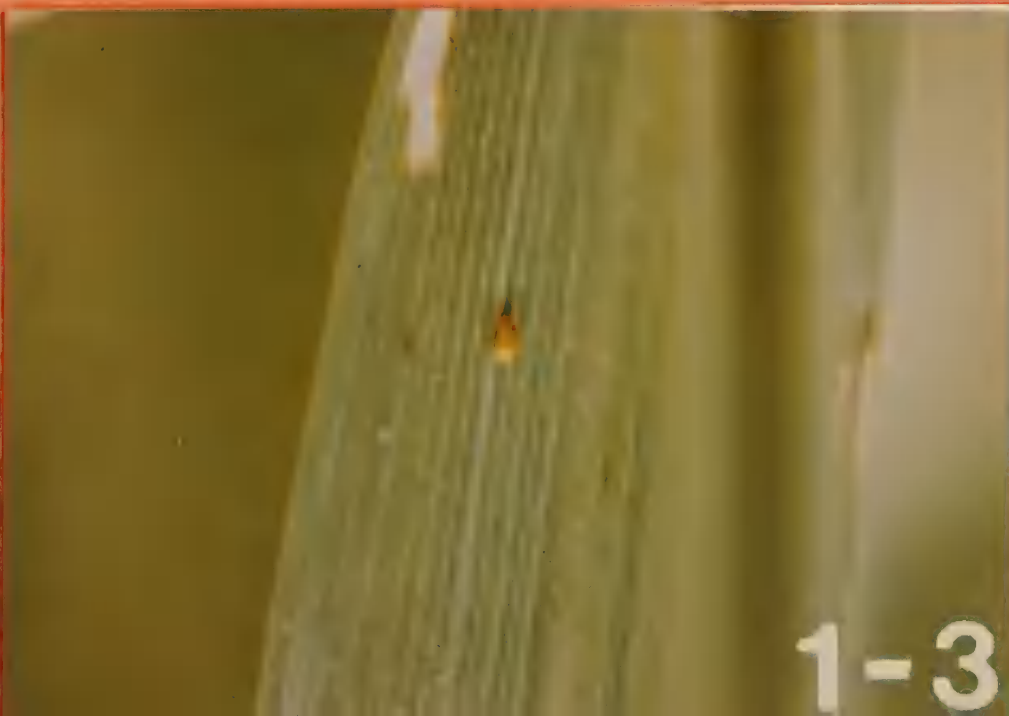
RELEASES OF NATURAL ENEMIES
Release Larval Parasites as Parasitized Larvae

- Step 1** When releasing CLB larval parasites, whether as adults or as parasitized larvae, it is essential that the release field not be plowed or otherwise tilled until after July 1 the following year. The CLB larval parasites remain in the soil from mid-summer until late the following spring. If the release site is tilled during this time, the parasites will be destroyed.
- Step 2** Parasitized CLB larvae will be transported in sealed styrofoam coolers or in suitable containers modified for their transportation. Release these larvae by removing the plant material containing the larvae and placing it on or among the host plants at the release site. Cut or pull release material coolers from the flats before placing it on or among host plants.
- Step 3** Use approximately 1,000 larvae at a general release site, and place a minimum of 5,000 to 6,000 larvae at a new insectary site.
- Step 4** Write the code number from each carton in block 9 of the FDWS.
- Step 5** Fill out blocks 10-23 and 28-34 on the FDWS. Draw a map of the release field and surrounding landmarks in block 29 of the FDWS. On this map, also indicate approximate release points in the field.
- Step 6** Return the white copy of the FDWS to NBCL, Niles, MI. Retain the yellow copy for your records, and forward the pink copy to your State project coordinator.

APPENDIX 1

Life Stages of the Cereal Leaf Beetle (CLB)

Introduction	Use this appendix to help identify life stages of CLB and plants damaged by CLB. The appendix includes photographs as well as narrative descriptions of the life stages of CLB. The number in brackets [] corresponds to the photograph number. The photographs are located in the back of this appendix.
<i>Oulema melanopus</i> (CLB) Adults [1-1], [1-2]	<p>The adult CLB is a serious pest of small grains (oats, barley, and wheat). It is 7-9 mm in length with blue-black elytra (wing cover), orange thorax, black head, and orange legs. This insect has been a pest of cereal crops throughout Europe and Asia since recorded history.</p> <p>The CLB has one generation a year. It overwinters as an adult in sheltered places (surface litter, straw stubble, tree bark, etc.) that give it protection from damaging temperatures. When spring temperatures exceed 50° F, the adults become active. They begin feeding on native grasses (timothy, quackgrass, orchard grass, etc.) and fall-planted grains (such as winter wheat and rye). During the early spring, adults go in and out of their overwintering sites, depending on the temperature. When temperatures reach 65° F, they begin to fly and mate. During this period, CLB densities may become quite heavy in preferred fields of fall-planted host crops.</p>
CLB Eggs [1-3]	After completing a period of feeding and mating, the females begin laying eggs. CLB eggs are usually laid singly on the upper leaf surface with the long axis parallel to the leaf veins. You may find early eggs on native grasses or fall-planted small grains. The hairiness of the leaf seems to affect the selection of the site where the female CLB lays eggs. Spring-planted grains (oats, barley, and spring wheat) are the preferred hosts, and as soon as these grains are in the seedling stage, the adult beetles will move into these fields and begin feeding and egg laying.
CLB Larvae [1-4], [1-5]	CLB eggs hatch in 1 - 3 weeks, depending on temperature. After hatching, the larvae then go through four larval instars. Larvae usually feed on the upper leaf surface and consume all of the chlorophyll-containing cells (mesophyll), leaving the translucent lower cuticle intact. As the larva feeds, its waste products are excreted to form a black, slimy fecal sac which covers its top and sides. Each time the larva molts, the fecal sac and exoskeleton are cast off and a "new" larva emerges. The fecal sac protects the larva from drying out and may ward off some parasites or predators.
CLB Feeding Damage [1-6]	The spring-planted grains are not the only fields that suffer from infestations of CLB. This field of winter wheat has been severely damaged. Note the flag leaves on these plants that are just beginning to head out. Most of these leaves have been stripped of their green, sugar-producing cells. An average of one larva per flag leaf will cause a 5-6 bushel loss in each acre of grain.





Block # 66830-000
1-800-101-0111
50th Central Plaza

APPENDIX 2

Natural Enemies of the Cereal Leaf Beetle (CLB)

Introduction	Use this appendix to help identify the natural enemies of CLB. The appendix includes photographs as well as narrative descriptions of the natural enemies. The number in brackets [] corresponds to the photograph number. The photographs are located in the back of this appendix.
<i>Anaphes flavipes</i> [2-1]	<p>The first parasite to be released and become established was the egg parasite, <i>Anaphes flavipes</i>. <i>A. flavipes</i> is a small mymarid wasp that lays its eggs inside the egg of the CLB. The females of this species have a clavate (clubbed) antenna, while the antenna of males is filiform (straight). <i>A. flavipes</i> may have from six to eight generations per year.</p> <p>The parasite eggs hatch almost immediately and the parasite larvae begin consuming the contents of the CLB egg. This red eye-spot stage is an advanced stage of development and indicates the beginning of the parasite pupal stage within the host egg. The parasite pupae then darken with age and take on a black body appearance just before they emerge. The bright yellow material is the parasite fecal material that is excreted when the parasite larvae pupate.</p>
<i>Diaparsis temporalis</i> [2-2]	<p><i>Diaparsis temporalis</i>, a member of the ichneumonid family, is a solitary larval parasite (one progeny per host). It appears to be well synchronized with its host; peak activity occurs at the time when CLB larvae are most abundant in the field. <i>D. temporalis</i> regularly parasitizes more than 90 percent of the CLB larvae at peak larval density in some areas. Even though this parasite has the best host synchrony, it is the slowest to disperse and build up since it is solitary and has only one generation per year.</p>
<i>Lemophagus curtus</i> [2-3]	<p><i>Lemophagus curtus</i>, another solitary ichneumonid, has two complete generations per year. In some cases, a portion of the second generation completes its development to become a partial third generation. <i>L. curtus</i> has parasitized in excess of 80 percent of the late season larvae in some areas. This wasp was the last of the three larval parasites to be established in the United States.</p>
<i>Tetrastichus julis</i> [2-4]	<p><i>Tetrastichus julis</i> is another of the three species of CLB larval parasites that have been introduced into the United States. It is a gregarious parasite (more than one progeny per host) with two generations per year. The numbers of this parasite found within one host may vary from 1-30 with the average being 4-6. One CLB larva collected in Pennsylvania contained 103 <i>T. julis</i> eggs and larvae. A member of the eulophid family, <i>T. julis</i> has attained parasitization rates exceeding 90 percent at or near peak larval density in many of the fields that have been sampled.</p>



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APPENDIX 3

Reporting Information Into the National Agricultural Pest Information System (NAPIS)

These instructions cover how to report releases and recoveries of cereal leaf beetle parasites into the NAPIS database. You must enter all release and recovery information into NAPIS. Direct questions on entering information to your State Cooperative Agricultural Pest Survey (CAPS) coordinator or PPQ Regional Survey Coordinator.

With many States entering data on the same program, it is important that each State uses the proper codes and enters all the required data. *Figures 16 and 17* are examples of worksheets that you can use to summarize data from the Field Data Worksheet for reporting data into NAPIS. Refer to the REF file for help in filling out the release and recovery worksheets. Use of these worksheets should decrease the time for data entry and reduce errors.

In addition to all the information collected during the recovery and release activities, you will need to know the longitude and latitude for each site. Also, you will need to know the Data Element Codes and Environmental Protection Agency (EPA) Codes. By using these codes listed in *Figure 18*, you will ensure data quality. Information access across State boundaries will be consistent. Ad hoc queries, reports, and computer mapping depend on all the proper codes being entered.

When reporting recovery information, remember that all identifications must be confirmed by the staff of the National Biological Control Laboratory (NBCL), Niles, Michigan, before you enter the species information into NAPIS. You should enter information into NAPIS immediately after sampling.

CLB Natural Enemy Release Worksheet for NAPIS Data Input

Observation Number										Observation Date										Data Source	
										1	9	9	3	0	5	2	6			1	1
(Local Determination)										Year/Month/Day										11-USDA-APHIS 13-State Dept. of Agric. 14-Univ./Extension	

State-County						EPA Site Code						Crop Life Stage				Crop Situation					
30111																					
(State & County FIPS Code)						(See REF-CROP Reference file)						(Leave Blank)				(Leave Blank)					

Location Coordinates												EPA Pest Code									
45°40'06"												105°53'01" ISAUAAHA									
(Latitude & Longitude of Find)												(Use Natural Enemy Code)									

Pest Life Stage		Pest Status				Survey Method				Quantification			
I6		38								1500			
(Natural Enemy Lifestage)		(Natural Enemy Status)				(See Listing in Manual)							

Descriptor Units			Total Units Checked			Positive Units			Observation Duration			Diagnostic Lab		
698														
									(Leave Blank)			(Leave Blank)		

Confirmation Method						Biocontrol Target																
						I	N	A	M	C	M	A										
(Leave Blank)																						

Notes																							

Figure 16: Example of a Release Worksheet for reporting data into NAPIS

CLB Natural Enemy Positive Recovery Worksheet for NAPIS Data Input

Observation Number	Observation Date	Data Source
1 9 9 4 0 7 0 6	1 9 9 4 0 7 0 6	1 1
(Local Determination)	Year/Month/Day	11-USDA-APHIS 13-State Dept. of Agric. 14-Univ./Extension

State-County	EPA Site Code	Crop Life Stage	Crop Situation
3 0 0 0 9			
(State & County FIPS Code)	(See REF-CROP Reference file)	(Leave Blank)	(Leave Blank)

Location Coordinates	EPA Pest Code
4 5 2 0 1 8 1 0 8 5 6 3 7	I S B T A B A
(Latitude & Longitude of Find)	(Use Natural Enemy Code)

Pest Life Stage	Pest Status	Survey Method	Quantification
I 5	+ B		1 0
(Natural Enemy Lifestage)	(Natural Enemy Status)	(See Listing in Manual)	

Descriptor Units	Total Units Checked	Positive Units	Observation Duration	Diagnostic Lab
6 8 3				
			(Leave Blank)	(Leave Blank)

Confirmation Method	Biocontrol Target
	I N A M C M A

Notes

Figure 17: Example of a Recovery Worksheet for reporting data into NAPIS

Data Element Codes	
Element	Code
1. Pest (Natural Enemy) Lifestage	
a. Larvae - use for parasitized CLB eggs or larvae	I6
b. Adult Parasites	I5
2. Pest (Natural Enemy) Status	
Positive (Present)	+
Negative (Not present in sample)	-
New or reintroduced in the State	2
New or reintroduced in the County	3
Known to be established	A
Not known to be established	B
3. Descriptor Unit	
Biological Agent Recovery	683
Beneficials Released at Site	698

EPA Codes	
Organism	Code
Natural Enemies	
Hymenoptera:Mymaridae <i>Anaphes flavipes</i>	ISBTABA
Hymenoptera:Ichneumonidae <i>Diaparsis temporalis</i>	ISAUAHA
Hymenoptera:Ichneumonidae <i>Lemophagus curtus</i>	ISAUAJA
Hymenoptera:Eulophidae <i>Tetrastichus julis</i>	ISAPAGA
Crop	
Barley - <i>Hordeum vulgare</i>	28063
Oats - <i>Avena sativa</i>	28062
Wheat - <i>Triticum spp.</i>	28065
Grasses (Hay)	28066
Pest	
Coleoptera:Chrysomelidae <i>Oulema melanopus</i>	INAMCMA

Figure 18: Data Element Codes and Environmental Protection Agency (EPA) Codes

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BIOLOGICAL CONTROL OF CEREAL LEAF BEETLE PROJECT MANUAL
Comment Sheet

Directions: Use this sheet to suggest an improvement or to identify a problem in the content of the manual. To mail, please follow the directions on the next page.

Description of problem (error, inconsistency, missing or insufficient information, etc.):

Description of improvement or recommended change (add attachments if necessary):

=====

Reason for improvement or change:

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USDA-APHIS-R&D
7340 Executive Way, Suite A
Frederick, MD 21701

Attn: Bruce Attavian